Ecology, 71(3), 1990, pp. 905–920

NUTRIENT SPATIAL HETEROGENEITY: EFFECTS ON COMMUNITY STRUCTURE, PHYSIOGNOMY, AND DIVERSITY OF STREAM ALGAE[1]

CATHERINE M. PRINGLE
Marine Science Institute, University of California, Santa Barbara, California 93106 USA

Abstract. Nutrient-diffusing substrata (sand-agar plates) were combined with an in situ, flow-through bioassay system to experimentally separate effects of substratum and water enrichment on algal communities in a phosphorus-poor stream in northern Michigan, USA. Glass slides and both control and fertilized sand-agar substrata (N:P $\approx$ 16) were exposed to low ambient nutrient levels in a phosphorus-limited stream (N:P $\approx$ 40) and to water amended with nitrogen and phosphorus (N:P $\approx$ 16).

Quantitative cell counts of periphyton taxa and microscopic examination of intact communities on sand-agar substrata indicated that motile biraphid diatom taxa (e.g., *Navicula* and *Nitzschia* spp.) responded to nutrients in both the water and substratum, attaining spatial dominance in the upper canopy. These upperstory taxa comprised >50% total algal biovolume on sand-agar substrata and appeared to interfere with the response of sessile understory taxa (e.g., *Achnanthes minutissima, Cocconeis placentula*) to inorganic nutrient amendments to the water. Sessile taxa did respond to inorganic nutrients added to the water when growing on glass slides, where algal communities lacked a dense motile upperstory, indicating that substratum type can influence the nature of periphyton taxon response to nutrients. Periphyton community physiognomy and interactions between taxa are interrelating factors regulating algal response to nutrients from different sources. In addition, the form of phosphorus enrichment (inorganic vs. organic) influenced algal growth, physiognomy, and taxon composition. Algal response to the form of phosphorus was dependent on the source of the nutrient (water vs. substratum) and substratum type.

Taxon diversity was greater on all nutrient treatments relative to controls on both substratum types. Diversity and species richness were highest on enriched substrata exposed to low ambient nutrient levels, relative to more homogeneous nutrient regimes where communities on enriched substrata were exposed to water amended with nutrients. Results indicate that nutrient spatial heterogeneity between substratum and water is a mechanism maintaining the species diversity of periphytic algae.

Key words: community structure; diatoms; diversity; motility; nutrients; periphyton; physiognomy; temperate stream.

INTRODUCTION

Aquatic systems are frequently classified as nitrogen or phosphorus limited on the basis of ambient nutrient levels and in situ bioassays of periphyton growth (e.g., Stockner and Shortreed 1978, Elwood et al. 1981*b*, Peterson et al. 1983, Triska et al. 1983, Grimm 1986, Pringle et al. 1986). Such information, however, does not reflect localized sources of nutrients that contribute to differences in the distribution and composition of attached algal communities (e.g., Pringle 1985*a*). Stream benthic habitats are mosaics of microenvironments that differ in chemical nature and are potentially limited by different nutrients. Nutrient spatial heterogeneity includes spatial variation in concentrations of: (1) the same nutrient, (2) different nutrients, and/or (3) different forms of the same nutrient (e.g., organic vs. inorganic).

This study examines algal response to spatial variation in nutrient concentrations in the water vs. the substratum. Attached algae possess a variety of characteristics and adaptations that may affect their ability to exploit nutrients from these different sources. Such characteristics include: presence of enzymes such as alkaline phosphatase (e.g., Kuenzler and Perras 1965, Wetzel 1981), mode of enzyme release (e.g., Healey 1973), heterotrophic abilities (e.g., Hellebust and Lewin 1977), and luxury consumption and storage capabilities (e.g., Fitzgerald and Nelson 1966, Rhee 1972).

The distinction between sessile understory taxa and loosely attached upperstory forms is useful when examining resource exploitation from the water vs. substratum. Locomotory capabilities of diatoms may also affect the position of a given taxon in the algal canopy and resource exploitation from different sources. If the microhabitat of an individual is defined as the area from which that individual obtains resources (Tilman 1982), the microhabitat of motile forms is larger than that of nonmotile taxa. Accordingly, diatom taxa may be distributed among three groups: (1) motile, biraphid

[1] Manuscript received 13 July 1988; revised 17 August 1989; accepted 20 August 1989.

diatoms capable of relatively rapid movement, (2) relatively sessile, monoraphid (and some biraphid) taxa capable of slow sluggish movement, and (3) sessile, araphid, stalked taxa (e.g., Pringle 1985*b*, Hudon and Legendre 1987).

An increasing number of studies have described the architecture of epibenthic algal communities (e.g., Allanson 1973, Hudon and Bourget 1981, 1983, Hoagland et al. 1982, Hoagland 1983, Korte and Blinn 1983, Hamilton and Duthie 1984, Pringle 1985*a*, Steinman and McIntire 1986). More recently, attention has been focused on algal physiognomic changes in response to environmental variations such as grazing (Steinman et al. 1987), light intensity (e.g., Hudon and Legendre 1987), and disturbance (Luttenton and Rada 1986). However, no studies have evaluated how nutrient differences between substratum and overlying waters influence attached algal communities.

The present study investigates how nutrients supplied from the water and the substratum determine periphyton succession, community structure, diversity, and physiognomy. This study was designed to test the hypothesis that there would be differences in the way different groups of diatoms, defined by position in the canopy and motility, responded to nutrient perturbations from different sources (substratum or water). The study secondarily examined: (1) the comparative response of algae to organic or inorganic phosphorus enrichment, (2) whether the response of individual periphyton taxa to nutrient enrichment is consistent among communities on different substratum types, and (3) if substratum specificity breaks down in response to nutrient enrichment.

Methods

Study site

Experiments were conducted in Carp Creek, a second-order, sand-bottomed stream located in northern Michigan on the University of Michigan Biological Station tract, Cheboygan County (45°33′ N, 34°40′ W). The stream is fed by a stable supply of cold groundwater and exhibits relatively constant discharge and temperature conditions ideal for in situ experiments. Chemically, the stream is nearly pristine and has very low concentrations of biologically important nutrients. Background molar N:P ratios are high (NO_3-N:PO_4-P [soluble reactive phosphorus] $\approx$ 40), relative to ratios determined by Redfield et al. (1963) and Rhee (1978). Previous studies have shown that algal growth is phosphorus limited (Pringle and Bowers 1984, Pringle 1987). For a more detailed description of the stream see Pringle (1985*a*, 1987).

Bioassay technique and experimental design

An integrated bioassay technique was designed (Pringle 1987) that combines a flow-through bioassay apparatus, modified from Peterson et al. (1983), with nutrient-diffusing substrata (Pringle and Bowers 1984). The bioassay apparatus (Fig. 1) was constructed from four Plexiglas cylinders, each held parallel to the water flow in a wooden frame. The frame was oriented so that cylinders were half filled with flowing water and was secured 0.35 m above the stream bottom by driving four wooden stakes (located at corners of the frame) into bottom sediments. Current velocity was measured with a Pygmy Gurley meter every 3 d throughout each experiment, at upstream and downstream ends of each cylinder. Water level fluctuations were recorded daily. Four Plexiglas baffles located in upstream ends of all cylinders insured turbulent mixing (Fig. 1). Artificial substrata (sand-agar substrata and glass slides) were secured in downstream ends of cylinders and placed so that substratum surfaces were parallel to the water flow.

Both control and nutrient-diffusing, sand-agar substrata were constructed according to methods presented by Pringle and Bowers (1984). Sand from the stream bed was washed and autoclaved to kill attached microorganisms and then consolidated with agar into plastic disposable petri dishes. Agar solutions were enriched with various concentrations of nitrate and phosphate (Table 1). Agar and sand were thoroughly mixed and the substratum was then scraped flush with the lip of each petri dish, creating a firm surface for periphyton colonization after cooling and solidification. Enriched substrata release nutrients in an exponentially decaying fashion (Pringle and Bowers 1984), similar to some natural nutrient-diffusing substrata (Elwood et al. 1981*a*).

Glass microscope slides were mounted in microscope slide holders (modified after the Catherwood diatometer of Patrick and Hohn [1956]) and three sets of six slides were secured in each cylinder, upstream of nutrient-diffusing substrata so that they would not be affected by nutrients released into the water from these substrata (Fig. 1). After incubation of a slide set in the stream, the two slides on outer sides of each set were discarded because they experienced dissimilar current regimes relative to other slides.

Nutrients were introduced into two of the cylinders by siphoning concentrated solutions into upstream ends from a 20-L carboy. The reservoir was encased in black plastic to prevent growth of photosynthetic organisms and/or light degradation of organic compounds. Nutrient solutions in the reservoir were renewed every 3 d with analytic grade chemicals. Rates of nutrient addition to cylinders were monitored and calibrated every day by adjusting valves in nutrient feeder lines. Dye additions at the upstream end of cylinders indicated thorough mixing by baffle systems. Water flow in cylinders appeared to be $\approx$80% that of adjacent stream waters.

To examine the response of periphyton to water and substratum enrichment, fertilized and unfertilized sand-agar substrata were exposed to both enriched and unen-

riched waters (Fig. 1, Table 1). In these experiments, each cylinder served as an experimental unit. Spatial interspersion of treatments (Hurlbert 1984) was not possible because randomized placement of enriched and unenriched substrata within the same cylinder would contaminate the overlying water and affect algae on unenriched substrata. The design assumes that conditions between cylinders were homogeneous outside of treatment effects. In support of this assumption (i.e., that cylinders are homogeneous experimental units): (1) a 0.9-m cross section of the stream was chosen that exhibited minimal variations in light intensities, sedimentation, current velocity, and depth between cylinder locations; (2) glass slide substrata were placed within all cylinders and retrieved in replicates of three on retrieval dates for sand-agar substrata, allowing simultaneous assessment of potential effects resulting from cylinder placement (two cylinders per treatment with respect to glass slides); (3) cylinders represent open microcosms that are continuously flushed with a well-mixed volume of water and which are delineated by mechanical barriers so that treatments that would otherwise affect each other are separated. Continuous immigration and emigration are permitted, thereby offsetting independent community evolution resulting from early differences, a trend commonly observed in closed systems such as aquaria (Whittaker 1961).

Substratum vs. water enrichment experiments can be mathematically expressed as a $(2)^2$ factorial main effects model, with no interaction term because of the lack of simultaneous replication of treatment combinations (Searle 1971):

$$Y_{ijk} = \mu + \alpha_i + \beta_j + E_{ijk},$$

where Y_{ijk} = estimate of individual observation, μ = overall effect, α_1 = effect due to unenriched substratum treatment, α_2 = effect due to enriched substratum treatment, β_1 = effect due to unenriched water treatment, β_2 = effect due to enriched water treatment, and E_{ijk} = unexplained variation, where k indexes replicates within each cylinder (k = 1–3). Substratum–water enrichment experiments were designed to test the null hypotheses: H_0:$\alpha_1 = \alpha_2$ and H_0:$\beta_1 = \beta_2$. The following notation is used to describe substratum and water enrichment treatment combinations (cf. Fig. 1, Table 1): (0 0) unenriched substratum, unenriched water; (0 +) unenriched substratum, enriched water; (+ 0) enriched substratum, unenriched water; (+ +) enriched substratum, enriched water.

Experiment I examined effects of both inorganic phosphorus (KH_2PO_4) and inorganic nitrogen ($NaNO_3$), while Experiment II examined effects of organic phosphorus (β-glycerophosphate, $C_3H_7O_6PNa_2 \cdot 5H_2O$) in combination with inorganic nitrogen (Table 1A, B). In both of these experiments, ambient phosphorus levels were increased fivefold and nitrate levels doubled. Ambient phosphorus levels were increased only slightly so that periphyton growth response to phosphorus enrichment of the water would not be so great as to mask potential interactions between water and substratum. Frequent monitoring of total phosphorus (TP) and PO_4-P in reservoir batches of concentrated β-glycerophosphate solution and water outflow from Plexiglas cylinders revealed that no degradation of organic P occurred before its addition to stream water in Experiment II.

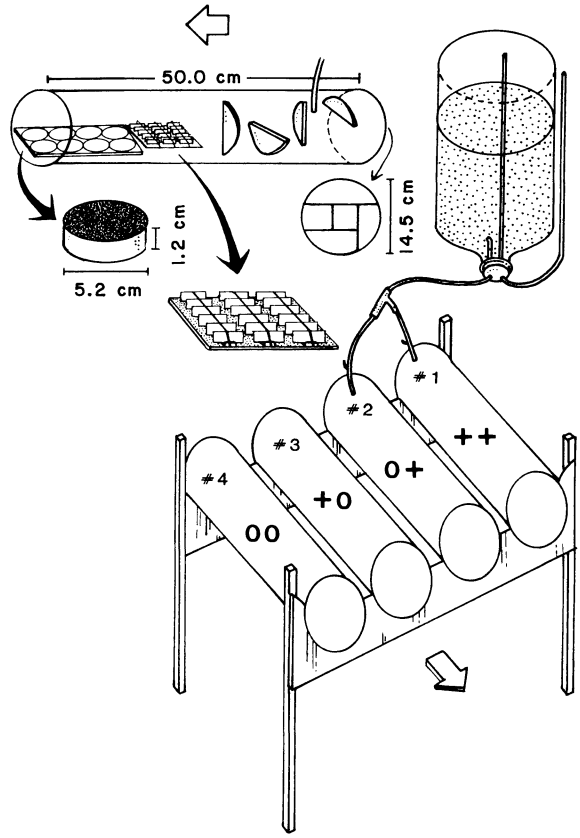

FIG. 1. Schematic diagram of periphyton bioassay system. Unshaded arrows denote current direction. Upper left: single Plexiglas cylinder showing placement of nutrient-diffusing substrata, glass slides, baffles, and nutrient feeder line. Enlargement of artificial substrata and inside view down length of cylinder (illustrating baffle arrangement) are shown directly below. Lower: bank of Plexiglas cylinders in wooden frame. Nutrients are added to upstream ends of cylinders 3 and 4 from 20-L carboy. Notation on cylinders refers to the treatment combination for substratum–water enrichment experiments where the first character refers to presence (+) or absence (0) of substratum enrichment and the second character refers to presence or absence of water enrichment.

To examine the response of periphyton on two different substratum types to water enrichment, glass slide substrata were compared to periphyton on sand-agar substrata. For glass slides, cylinders 1 and 2 (Fig. 1) served as enriched replicate treatments, while cylinders 3 and 4 served as unenriched replicate treatments.

Previous studies in Carp Creek have indicated that invertebrate grazers and retreat builders can significantly affect periphyton standing crop and/or species

TABLE 1A, B. Experimental design and corresponding nutrient regimes for treatments in Experiments I and II for sand-agar plates and glass slide substrata. Values are expressed in μmol/L, with μg/L in parentheses below.

(A) Experiment I: inorganic phosphorus

Treatment*		Substratum			Water		
Sand-agar plates	Glass slides	NO_3-N	PO_4-P†	N:P	NO_3-N	PO_4-P†	N:P
(0 0)	(0 0)	0.00	0.00	···	2.95 (41.34)	≤0.08 (≤2.50)	≥37
(+ 0)	···	8.00×10^6	0.50×10^6	≈16	2.95 (41.34)	≤0.08 (≤2.50)	≥37
(0 +)	(0 +)	0.00	0.00	···	5.71 (80.00)	0.40 (12.50)	≈14
(+ +)	···	8.00×10^6	0.50×10^6	≈16	5.71 (80.00)	0.40 (12.50)	≈14

(B) Experiment II: organic phosphorus

Treatment		Substratum			Water		
Sand-agar plates	Glass slides	NO_3-N	P‡	N:P	NO_3-N	P‡	N:P
(0 0)	(0 0)	0.00	0.00	···	3.65 (51.10)	≤0.08 (≤2.50)	≥46
(+ 0)	···	1.5×10^7	0.90×10^6	≈16	3.65 (51.10)	≤0.08 (≤2.50)	≥46
(0 +)	(0 +)	0.00	0.00	···	5.71 (80.00)	0.40 (12.50)	≈14
(+ +)	···	1.5×10^7	0.90×10^6	≈16	5.71 (80.00)	0.40 (12.50)	≈14

* (0 0): unenriched substratum, unenriched water; (0 +): unenriched substratum, enriched water; (+ 0): enriched substratum, unenriched water; (+ +): enriched substratum, enriched water.
† PO_4-P = KH_2PO_4.
‡ P = $C_3H_7O_6PNa_2 \cdot 5H_2O$.

composition (Pringle 1979, 1985*a*). Artificial substrata within cylinders were, therefore, checked daily to remove invertebrates and retreats. Three replicate plates and four glass slides were collected randomly from each cylinder at approximately weekly intervals over a 3-wk period. Visibly thick periphyton mats formed on artificial substrata in Experiment II after only 19 d, at which time the experiment was terminated so that final collections could be made before sloughing. Ambient water chemistry was monitored every 3 d throughout experiments and nutrients were analyzed on a Technicon II autoanalyzer. PO_4-P was measured in filtered samples using an automated molybdenum blue technique (Murphy and Riley 1962) and TP was measured in unfiltered samples using this technique preceded by acid hydrolysis. Filtered samples were also analyzed for (NO_2+NO_3)-N and NH_4-N using an automated cadmium reduction technique (Armstrong et al. 1967) and the phenate method (APHA 1980), respectively.

Periphyton analyses

After stream incubation, sand-agar substrata were cored with a cork borer (1.2 cm diameter × 1.2 cm length). Two cores were removed; one core was temporarily refrigerated for later nutrient analysis (results presented in Pringle 1987). The top 3 mm layer of the second core was removed and sliced in half. One section was fixed in phosphate-buffered glutaraldehyde for scanning electron microscopy analysis. The remaining section was further sectioned into three, 1 mm thick horizontal layers and squash mounted on slides with Hyrax for qualitative analysis of community physiognomy under a light microscope. The top 3 mm of substratum remaining in petri plates were then removed with a razor blade. Microscopic inspection indicated that few algal cells were located below 2 mm. The layer was placed in a 200-mL beaker, diluted to 100 mL with distilled water, mixed for 10 min with a magnetic stirrer, and then sonicated for 5 min. This procedure resulted in almost complete periphyton separation from sand grains as evidenced by microscopic scanning of residual grains.

Four duplicate glass slides per cylinder were placed into separate 200-mL beakers, each containing 50 mL of distilled water. In the laboratory, one of the four slides was used to examine the three-dimensional structure of the periphyton community. This slide was immediately examined under a light microscope and subsequently air-dried. Crude yet permanent slide mounts were created by adding drops of Hyrax at three equidistant points on one side of the slide, topping each drop with a coverslip, followed by heating to create a burn mount. The three remaining slides per cylinder were carefully scraped with a razor blade followed by

a fine brush lubricated with distilled water. Scrapings were diluted to 100 mL with distilled water and the solution was homogenized for 5 min with a magnetic stirrer.

Chlorophyll *a* samples (2.0 mL) were taken separately from the suspended periphyton homogenate obtained for both glass slides and sand-agar substrata. Samples were filtered (0.45-μm mesh Millipore filter), extracted in 90% buffered acetone and $MgCO_3$ and stored frozen in dark bottles for up to 1 wk, at which time they were analyzed with a Turner III fluorometer. Chlorophyll *a* values were corrected for phaeopigments (Holm-Hansen et al. 1965) and expressed as milligrams per square centimetre of substratum surface.

Three subsamples (0.1–1.2 mL) were also taken from the suspended algal homogenate derived from substrata and placed upon individual glass coverslips to air-dry. Permanent slide mounts were subsequently prepared with Hyrax for all experiments. This procedure does not result in clearing of cells as do conventional techniques (Patrick et al. 1954, Hohn and Hellerman 1963), but discriminates between live and dead cells. Diatom taxa comprised at least 95% of the periphyton biovolume in all samples and counts were restricted to diatom taxa. A compound microscope (1000×) was used to enumerate live cells containing protoplasm. At least 500 diatoms per sample were enumerated, with a resulting count of ≈1500 frustules per treatment on each sampling date. Cell volumes of specific taxa were estimated by entering mean diatom measurements into geometric equations that best describe the three-dimensional shape of the frustule (Gruendling 1971). A minimum of 25 cells per diatom taxon was used to determine mean dimensions.

Scanning electron microscopy was used to qualitatively examine intact periphyton communities upon substratum sections (removed from sand-agar substrata with a cork borer, as described above). Sections were fixed in phosphate-buffered glutaraldehyde and then dehydrated in a graded series of increasing concentrations of ethanol. Sections were subsequently air-dried after transfer to an absolute ethanol solution and then mounted with epoxy glue on aluminum stubs. Samples were pulse coated with 20 nm of gold-palladium and observed with a scanning electron microscope.

A two-way ANOVA (Sokal and Rohlf 1981) tested the null hypothesis that substratum and water enrichment each had no significant effect with respect to chlorophyll *a* and periphyton biovolume. If the hypothesis was rejected, a Student-Newman-Keuls test was used to compare treatment means on given retrieval dates. Treatment duplicates were pooled to determine treatment means. In some instances, multiple comparisons of regression coefficients describing these parameters through time were made using a simultaneous test procedure (Sokal and Rohlf 1981). Species diversity was calculated using the Shannon-Wiener informational index (Pielou 1966). Unless otherwise indicated, the criteron for significance for all statistical tests was $P < .05$.

TABLE 2. Selected chemical and physical variables measured in Carp Creek during bioassay Experiments I (31 August–22 September 1983) and II (5 July–24 July 1984). Data are means ± SD.

Variable*	Unit	Experiment I	Experiment II
SRP (PO_4-P)	μmol/L	0.08 ± 0.02 (n = 5)	0.08 ± 0.04 (n = 5)
TP	μmol/L	0.26 ± 0.08 (n = 4)	0.39 ± 0.08 (n = 4)
(NO_2 + NO_3)-N	μmol/L	2.95 ± 0.22 (n = 5)	3.65 ± 0.98 (n = 6)
NH_4-N	μmol/L	4.11 ± 0.61 (n = 5)	4.62 ± 1.63 (n = 3)
N:P	...	≥36.88 (n = 5)	≥45.62 (n = 5)
Temperature	°C	10.10 ± 0.80 (n = 21)	11.50 ± 1.20 (n = 18)

* SRP = soluble reactive phosphorus, TP = total phosphorus.

RESULTS

Mean current velocities within cylinders ranged between 18 and 20 cm/s ($\bar{X}$ ± SD = 19.0 ± 0.8 cm/s, n = 20). No significant differences in current velocities existed between cylinders (Student's t tests). Water level was always within 0.5 cm of the mean gauge reading. Selected chemical and physical variables measured in Carp Creek during bioassay experiments are given in Table 2. Water temperatures typically remained low ($\bar{X}$ = 10.2°C), however, temperatures in the latter half of Experiment II climbed to an unusual 12.7°. Carp Creek exhibited low levels of soluble reactive phosphorus (PO_4-P) throughout all experiments, with a mean summer concentration of 0.07 μmol/L.

Periphyton growth response

Total periphyton growth response (chlorophyll *a*, total biovolume) to water vs. substratum enrichment on sand-agar plates for Experiments I and II is presented elsewhere (Pringle 1987). The dynamics of phosphorus distribution in nutrient-diffusing substrata is also presented in Pringle (1987). Chlorophyll *a* and total diatom biovolume on glass slides (Table 3) and sand-agar substrata (Pringle 1987) exposed to inorganic nitrogen and phosphorus (Experiment I) were significantly greater than on controls after 22 d. Enrichment of water with organic phosphorus (β-glycerophosphate) and inorganic nitrogen (Experiment II) had no significant effect on either chlorophyll *a* or total diatom biovolume accrual on glass slides (Table 3) or sand-agar substrata (Pringle 1987).

Response of individual periphyton taxa

Diatoms comprised >95% of the total periphyton biovolume on artificial substrata in all experiments and

TABLE 3. Chlorophyll *a* levels and total diatom biovolume (means ± SD in both cases) on glass slides after 22 d in situ exposure to ambient stream nutrient levels and to water enriched with phosphorus and nitrogen.

Treatment	Experiment I		Experiment II	
	Chl *a* (mg/m^2)	Biovolume (10^7 $\mu m^3/cm^2$)	Chl *a* (mg/m^2)	Biovolume (10^7 $\mu m^3/cm^2$)
(0 0) Control	4.06 ± 0.37	7.77 ± 0.63	3.94 ± 0.94	8.70 ± 0.68
(0 +) Enriched	7.13 ± 1.79	14.36 ± 1.02	4.07 ± 0.89	8.24 ± 0.51
P	<.05	<.05	NS	NS

* In Experiment I, glass slides were exposed to water enriched with inorganic phosphorus and nitrogen, while in Experiment II water was enriched with organic phosphorus and inorganic nitrogen.

42 species were identified. Taxon-specific effects were evaluated for five major taxa that together comprised >75% of total diatom biovolume: (1) *Achnanthes minutissima* Kutz., (2) *Cocconeis placentula* Ehr., (3) *Navicula* spp., (4) *Nitzschia* spp., and (5) *Opephora martyi* Herib.

Achnanthes minutissima.—In Experiment I (inorganic P and N), *A. minutissima* biovolume was significantly greater on enriched sand-agar substrata (+ 0, + +) relative to unenriched substrata (0 0, 0 +) in both the presence and absence of water enrichment after 22 d (Student-Newman-Keuls test). This taxon did not respond significantly to water enrichment when growing on unenriched sand-agar substrata (0 +). Combined influences of nutrients supplied from the substratum and water exceeded the sum of individual effects, implying a synergistic effect (Table 4). While *A. minutissima* did not respond to inorganic nutrients in the water when growing on sand-agar substrata, it did respond significantly to this treatment when growing on glass slides after 16 and 22 d (Table 5A).

In Experiment II (organic P, inorganic N) after 15

TABLE 4. Cell biovolumes (means ± SD) for selected diatom taxa on sand-agar substrata in response to different treatment combinations in Experiments I and II.*

(A) Experiment I

Taxon	No. of days incubated	Treatment* (0 0)	(0 +)	(+ 0)	(+ +)
		Cell biovolume (10^7 $\mu m^3/cm^2$)			
Achnanthes minutissima	16	1.88 ± 0.38	1.57 ± 0.29	2.85 ± 0.99	3.64 ± 0.37
	22	2.22 ± 0.54	2.64 ± 0.24	3.58 ± 0.56	6.74 ± 0.37
Cocconeis placentula	16	0.88 ± 0.23	0.95 ± 0.33	2.77 ± 1.01	1.14 ± 0.51
	22	0.84 ± 0.41	0.71 ± 0.17	2.94 ± 0.33	2.26 ± 0.32
Navicula spp.	16	2.76 ± 1.09	6.18 ± 0.74	15.58 ± 5.10	15.63 ± 3.81
	22	3.37 ± 0.99	9.92 ± 1.10	28.68 ± 5.20	35.88 ± 9.14
Nitzschia spp.	16	1.50 ± 0.40	2.69 ± 0.32	10.31 ± 4.79	8.58 ± 3.06
	22	2.11 ± 0.31	3.63 ± 0.48	16.10 ± 4.22	18.61 ± 5.50
Opephora martyi	16	1.47 ± 0.76	2.63 ± 1.29	8.48 ± 3.77	2.99 ± 0.82
	22	2.07 ± 0.44	2.88 ± 1.12	7.19 ± 0.80	14.45 ± 1.21

(B) Experiment II

Taxon	No. of days incubated	Treatment* (0 0)	(0 +)	(+ 0)	(+ +)
Achnanthes minutissima	15	5.54 ± 0.65	2.80 ± 0.03	2.08 ± 0.10	2.21 ± 0.75
	19	20.76 ± 2.22	19.30 ± 3.24	10.32 ± 1.60	8.19 ± 0.98
Cocconeis placentula	15	2.24 ± 0.28	0.74 ± 0.09	2.35 ± 1.79	1.34 ± 0.10
	19	6.14 ± 3.35	9.88 ± 6.62	9.00 ± 4.56	4.44 ± 1.20
Navicula spp.	15	4.67 ± 1.40	4.02 ± 0.16	17.03 ± 5.51	20.82 ± 4.48
	19	3.19 ± 0.84	15.67 ± 11.12	31.47 ± 3.68	46.68 ± 5.56
Navicula pelliculosa	15	0.31 ± 0.23	1.06 ± 0.45	8.67 ± 2.36	11.48 ± 2.58
	19	0.99 ± 0.59	1.38 ± 0.66	9.66 ± 1.39	24.74 ± 2.54
Nitzschia spp.	15	3.42 ± 1.32	8.87 ± 4.84	24.52 ± 8.80	32.72 ± 11.50
	19	10.58 ± 5.82	16.48 ± 3.36	52.19 ± 5.99	55.54 ± 3.98
Opephora martyi	15	1.88 ± 0.70	1.48 ± 0.54	5.09 ± 1.20	6.47 ± 1.26
	19	1.60 ± 1.48	5.84 ± 1.10	6.44 ± 2.77	10.68 ± 1.83

* (0 0): unenriched substratum, unenriched water; (0 +): unenriched substratum, enriched water; (+ 0): enriched substratum, unenriched water; (+ +): enriched substratum, enriched water. In Experiment I water was enriched with inorganic phosphorus and nitrogen while in Experiment II water was enriched with organic phosphorus and inorganic nitrogen.

TABLE 5A, B. Cell biovolumes (means ± SD) for selected diatom taxa growing on glass slides after various exposure periods to ambient stream nutrient levels (control) and to water amended with nitrogen and phosphorus (enriched).*

(A) Experiment I

Taxon	No. of days incubated	Treatment* Control (0 0)	Treatment* Enriched (0 +)	P
		Cell biovolume (10^7 $\mu m^3/cm^2$)		
Achnanthes minutissima	16	0.20 ± 0.02	0.38 ± 0.07	<.05
	22	2.76 ± 0.64	10.10 ± 4.70	<.05
Cocconeis placentula	16	8.32 ± 0.71	9.60 ± 0.44	NS
	22	70.02 ± 7.29	95.59 ± 13.23	<.05
Navicula spp.	16	0.56 ± 0.15	0.41 ± 0.20	NS
	22	0.50 ± 0.38	7.52 ± 1.60	<.05
Nitzschia spp.	16	0.18 ± 0.06	0.24 ± 0.17	NS
	22	1.02 ± 0.79	5.43 ± 1.55	<.05
Opephora martyi	16	0.17 ± 0.05	0.47 ± 0.16	<.05
	22	0.29 ± 0.16	1.98 ± 0.82	<.05

(B) Experiment II

Taxon	No. of days incubated	Treatment* Control (0 0)	Treatment* Enriched (0 +)	P
Achnanthes minutissima	15	0.28 ± 0.01	0.17 ± 0.02	<.05
	22	2.74 ± 0.51	2.51 ± 0.38	NS
Cocconeis placentula	15	19.48 ± 2.86	22.64 ± 4.42	NS
	22	78.41 ± 4.01	74.92 ± 7.33	NS
Navicula spp.	15	0.29 ± 0.19	0.22 ± 0.08	NS
	22	0.69 ± 0.28	0.58 ± 0.14	NS
Nitzschia spp.	15	0.39 ± 0.09	0.48 ± 0.21	NS
	22	1.14 ± 0.38	1.36 ± 0.29	NS
Opephora martyi	15	0.13 ± 0.05	0.15 ± 0.01	NS
	22	0.32 ± 0.12	0.41 ± 0.15	NS

* (0 0): unenriched substratum, unenriched water; (0 +) unenriched substratum, enriched water. In Experiment I water was enriched with inorganic phosphorus and nitrogen while in Experiment II water was enriched with organic phosphorus and inorganic nitrogen.

d, the biovolume of *A. minutissima* was significantly less for all nutrient treatments (0 +, + 0, + +) relative to controls (0 0) on sand-agar substrata. After 19 d, differences between controls and substratum-enriched treatments were still significantly different and the negative response of this taxon to combined influences of substratum and water enrichment was about equal to the sum of individual effects, implying an additive negative effect (Table 4). *A. minutissima* did not respond to enrichment of the water with organic phosphorus and inorganic nitrogen when growing on glass slides (Table 5B).

Cocconeis placentula.—This taxon also did not respond significantly to water enrichment with inorganic nutrients when growing on unenriched sand-agar substrata (0 +; Table 4). Both enriched substratum treatments (+ 0, + +) exhibited significantly greater biovolumes of *C. placentula* relative to unenriched substratum treatments (0 0, 0 +) after 22 d (Student-Newman-Keuls test).

On glass slides, *C. placentula* responded significantly to enrichment of the water with inorganic nutrients after 22 d (Table 5A) in contrast to its lack of response on sand-agar substrata (Table 4; [0 +] treatment). The relative biovolume abundance of *C. placentula* increased on control glass slides through time, reaching ≈90% after 22 d. Though *C. placentula* on glass slides increased in response to enrichment, its relative biovolume abundance decreased relative to controls. In contrast, control sand-agar substrata exhibited consistently low biovolume proportions of *C. placentula* on all sampling dates. Relative biovolume abundance of this species also decreased through time on sand-agar substrata exposed to enrichment.

In Experiment II, *C. placentula* on sand-agar substrata did not exhibit significant biovolume differences between treatments after 22 d (Table 4). This taxon also did not respond to water enrichment with organic phosphorus and inorganic nitrogen when growing on glass slides (Table 5B).

Navicula and *Nitzschia* spp.—Both of these genera showed a significantly greater response on enriched sand-agar substrata relative to unenriched substrata in both the presence and absence of water enrichment on the last two sampling dates of Experiments I and II (Table 4). These taxa exhibited an additive response to combined substratum and water enrichment in both experiments. When growing on glass slides (Table 5), *Navicula* and *Nitzschia* spp. exhibited a significant response to water enrichment affter 22 d in Experiment

I (inorganic P and N), however, they did not respond to water enrichment in Experiment II (organic P and inorganic N). Both of these genera maintained similar relative biovolume proportions through time on sand-agar substrata exposed to both enriched (0 +) and unenriched water (0 0) and on glass slides exposed to enriched water. Percentage biovolume decreased through time on control glass slides, however. In Experiment I (inorganic P and N), *Navicula pelliculosa* Breb. ex Kutz. was not recorded, however, in Experiment II (organic P, inorganic N) this species comprised up to 50% of the biovolume of total *Navicula* spp. on enriched substratum treatments (+ 0, + +) after 19 d and was therefore examined separately. *N. pelliculosa* did not show a significant response to water enrichment (0 +), however, it did respond significantly to substratum enrichment (+ 0) and to combined effects of substratum and water enrichment (+ +) after 16 and 22 d, relative to unenriched substrata (0 0, 0 +), exhibiting increases of 5- and 12-fold, respectively (Table 4). This species was not recorded on glass slide substrata.

Opephora martyi.—This taxon exhibited no significant differences in biovolume between control (0 0) and water-enriched (0 +) treatments after 16 and 22 d on sand-agar substrata in Experiment I. Biovolume was significantly greater on enriched substratum treatments (+ 0, + +) after 22 d (Table 4). Combined influences of substratum and water enrichment exceeded the sum of individual treatment effects after 22 d. On glass slides, *O. martyi* exhibited significantly greater biovolume when exposed to water enrichment after 16 and 22 d in Experiment I (Table 5A).

In Experiment II, *O. martyi* biovolume was significantly greater on all sand-agar substratum nutrient treatments (0 +, + 0, + +) relative to controls (0 0) after 19 d (Table 4). On glass slides, however, *O. martyi* did not respond significantly to water enrichment on any of the sampling dates (Table 5B).

To examine broader functional responses, algal taxa were grouped into categories based on number of raphes and potential for motility (Fig. 2): (1) araphid (sessile, stalked taxa), (2) monoraphid (relatively sessile taxa but capable of slow sluggish movement), and (3) biraphid (motile taxa capable of relatively rapid movement [with some exceptions, e.g., *Navicula pelliculosa*]).

In Experiment I, biraphid taxa on sand-agar substrata responded significantly to separate influences of water (0 +) and substratum enrichment (+ 0) with inorganic P and N after 16 and 22 d (Fig. 2A). Mono- and araphid taxa did not respond significantly to water enrichment (0 +), but they did show a significant response to substratum enrichment (+ 0) on these last two sampling dates. On glass slides, however, all three groups responded significantly to enrichment of the water with inorganic nutrients. After 22 d, mean (±SD) diatom biovolume of araphid taxa on control slides was $0.72 \pm 0.30 \times 10^6$ $\mu m^3/cm^2$, vs. $2.76 \pm 1.09 \times 10^6$ $\mu m^3/cm^2$ on slides exposed to nutrients. Biovolume of monoraphid taxa on control slides after 22 d was $73.91 \pm 7.00 \times 10^6$ $\mu m^3/cm^2$ vs. $121.23 \pm 11.65 \times 10^6$ $\mu m^3/cm^2$ in nutrient treatments. Biovolume of biraphid taxa on control slides after 22 d was $3.07 \pm 1.40 \times 10^6$ $\mu m^3/cm^2$, vs. $19.59 \pm 3.97 \times 10^6$ $\mu m^3/cm^2$ in response to nutrient amendments.

In Experiment II, biraphid and araphid taxa on sand-agar substrata responded significantly to separate influences of water and substratum enrichment with organic P and inorganic N (Fig. 2B). Monoraphid taxa did not respond significantly to either substratum or water enrichment.

Relatively sessile taxa predominated on glass slides, while motile taxa predominated on sand-agar substrata. Relative proportions of motile taxa on both glass slides and sand-agar substrata increased in response to water enrichment, relative to controls.

Community succession and physiognomy

In both experiments, *Opephora martyi* and *Navicula* spp. predominated early in colonization on sand-agar substrata. Relative biovolumes of *O. martyi* decreased through time on all treatments. At the end of experiments, enriched substratum treatments (+ +, + 0) were dominated by *Navicula* and *Nitzschia* spp., which together comprised from 62 to 67% of the total biovolume. Scanning electron and light microscope observations revealed that these taxa formed a thick upperstory mat on enriched substrata after the second sampling date. In Experiment I, the mat was dominated by *Navicula cryptocephala* Kutz., *N. cuspidata* (Kutz.) Kutz. var. *cuspidata, N. graciloides* A. Mayer, *N. tripunctata* (O. G. Mull.) Bory, *Nitzschia palea* (Kutz.) W. Sm., and *N. linearis* W. Sm., and was underlain by sessile attached forms such as *Opephora martyi* and *Achnanthes* spp.

In Experiment II, *Navicula pelliculosa* was the dominant naviculoid on enriched substrata, forming an almost continuous film, one cell thick, over the substratum (Fig. 3). This small naviculoid was present in significantly lower numbers ($P < .01$) on unenriched substratum treatments and was not even recorded in Experiment I. Individual cells of *N. pelliculosa* were enclosed by a mucilage pellicle that rendered cells immobile despite possession of two raphes. Light microscope observations revealed that the mat formed a thin mucilaginous skin over underlying sand-agar substratum and associated sessile flora (Fig. 4A). Motile biraphid diatoms such as *Nitzschia amphibia* Grun., *N. filiformis* (W. Sm.) Hust., *N. palea,* and lesser numbers of *Navicula* spp. formed an upperstory community overlying this mat (Fig. 4B).

In both Experiments I and II, upperstory biraphid diatoms predominated in enriched treatments on sand-agar substrata regardless of the source of enrichment, exhibiting significantly higher biovolumes and rates of biovolume accrual relative to controls. Biraphid taxa also exhibited significantly greater rates of biovolume

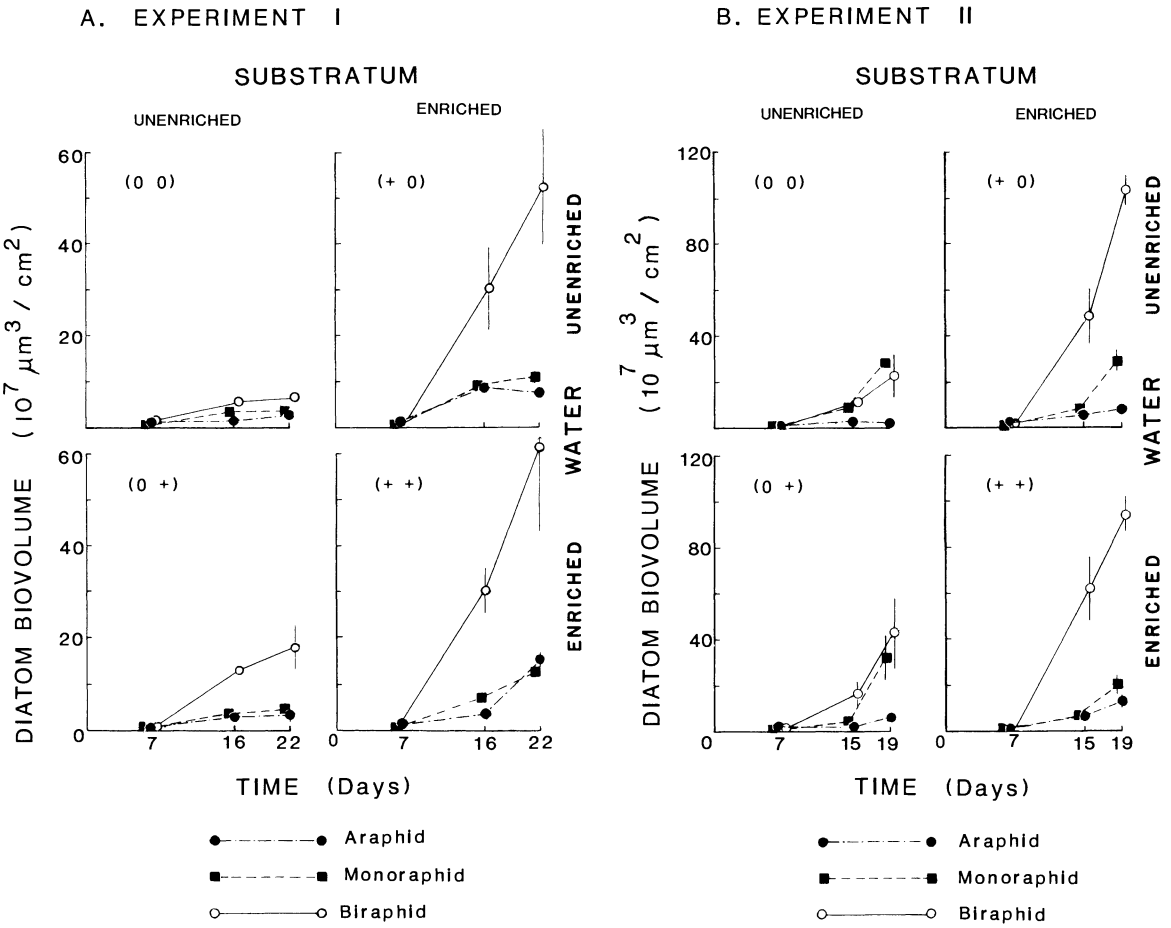

FIG. 2. Diatom biovolume accrual (means ± SD) of araphid, monoraphid, and biraphid diatoms on sand-agar substrata in response to different treatment combinations for Experiment I (inorganic N, inorganic P) and Experiment II (inorganic N, organic P). Characters in parentheses in data field indicate whether nutrient enrichment was present (+) or absent (0) in the substratum (first character) or in the water (second character).

accrual (between 7 and 22 d) than araphid and monoraphid taxa on all nutrient treatments in Experiment I; however, no significant difference existed between accrual rates of these functional groups on controls (Fig. 2A). In Experiment II, biraphid taxa exhibited significantly greater rates of biovolume accrual than araphid and monoraphid taxa on substratum-enriched treatments only (+ 0, + +; Fig. 2B). Mean (±SD) cell volume of motile taxa was 640.38 ± 770.10 μm^3 (n = 27; range = 118.34–3500.00 μm^3), while cell volume of relatively sessile and nonmotile taxa, combined, was 213.71 ± 151.11 μm^3 (n = 23; range = 52.13–996.60 μm^3).

Taxon diversity

Diatom taxon diversity (H') and richness (S) values were averaged for the three sand-agar substrata collected per treatment on all retrieval dates for both experiments (Fig. 5A, B). Diatom taxon diversity was significantly greater than controls for all enrichment treatments and greatest for communities developing on enriched substrata exposed to low ambient nutrient levels. Taxon diversity and richness of diatoms decreased from 7 to 19 d in all treatments with the exception of the (+ 0) treatment in Experiment I in which both increased significantly through time.

Mean (±SD) diatom taxon diversity on glass slides in Experiment I after 22 d was 1.92 ± 0.04 for water-enriched treatments and 1.50 ± 0.14 on controls. Species richness was 15.83 ± 1.17 and 14.50 ± 2.07 for these respective treatments. Sand-agar substrata exhibited significantly greater taxon diversity and taxon richness than glass slides in both experiments. Mean taxon diversity was also significantly greater for water-enriched treatments (0 +) than for controls (0 0) on both substratum types.

DISCUSSION

Periphyton growth response

The form of phosphorus enrichment (inorganic vs. organic P) influences algal growth and taxon compo-

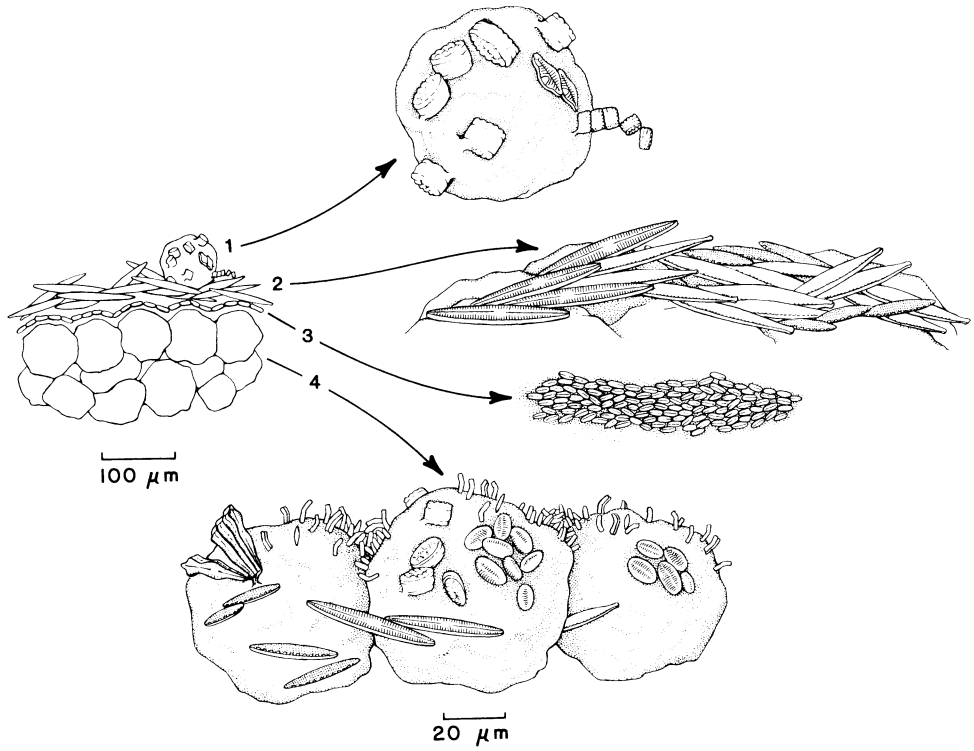

FIG. 3. Schematic cross section of periphyton community on sand-agar substratum enriched with β-glycerophosphate (Experiment II) after 19 d of stream incubation. Canopy layers in the periphyton mat include: (1) bed-load sand grains (and associated microflora) that have been deposited on the surface of sand-agar substrata and associated microflora. Stalked cells of *Opephora martyi* dominate the sand grain flora along with smaller numbers of adnate *Achnanthes lanceolata* and the stalked, chain-forming diatom *Fragilaria pinnata*; (2) thick upperstory mats of motile biraphid taxa dominated by patches of *Navicula tripunctata, Nitzschia amphibia,* and *N. filiformis*; (3) mucilaginous layer of *Navicula pelliculosa*; (4) underlying sand-agar matrix dominated by sessile and relatively sessile taxa (*Achnanthes minutissima, Cocconeis placentula, Gomphonema* spp., *Opephora martyi*) and smaller numbers of motile forms (*Navicula* and *Nitzschia* spp.).

sition. Substratum type may influence the nature of the response. Periphyton on both glass slides (Table 3) and sand-agar substrata (Pringle 1987) responded to inorganic P additions, while addition of organic P to the water caused no significant growth response on either substratum type. Since algal ability to use organic P is dependent on the activity of alkaline phosphatase enzymes (Koshland 1965, Kuenzler and Perras 1965, Patni et al. 1977) that are localized at the cell surface and extracellularly released in many taxa (Healey 1973), it is hypothesized that the overall lack of periphyton response to enrichment of water with organic P is because attached algae are poorly adapted for direct enzymatic uptake from overlying water. The positive growth response of periphyton to organic P within the substratum suggests that extracellular release of phosphatase enzymes may be a more effective means of exploiting organic P sources in the substratum (Pringle 1987). The proximity of periphytic diatoms to the underlying substratum would facilitate uptake of macromolecules bound or adsorbed to the sediment by extracellular enzymatic secretion. The implications of enzyme release are discussed later with respect to sessile vs. motile growth forms of algae. It is also possible that organic P within the substratum is transformed to inorganic P via bacterial degradation (Pringle 1987).

Resource exploitation and community physiognomy: response of individual periphyton taxa

The same taxa growing on different substratum types responded differently to water enrichment with inorganic nutrients. While all major taxa on glass slides responded to water enrichment in Experiment I, several major taxa exhibited no significant response on sand-agar substrata. Periphyton communities on sand-agar substrata exhibited more complex community physiognomy and significantly greater periphyton biomass, taxon richness, diversity, and evenness of taxon distribution. Interactions between taxa in these more complex communities can affect responses of individual taxa. Such interactions may mask or interfere with individual taxon response to nutrient addition that might be apparent in the less diverse and spatially complex community found on glass slides. The abundance of upperstory motile taxa (e.g., *Navicula* and *Nitzschia* spp.) on sand-agar substrata may negatively affect nu-

trient diffusion rates from overlying waters experienced by relatively sessile understory taxa (e.g., *Achnanthes minutissima, Cocconeis placentula, Opephora martyi*). This would explain the lack of response of these three taxa to water enrichment with inorganic nutrients when growing on sand-agar substrata (Table 4) and their positive response when growing on glass slides (Table 5).

The main differences in community physiognomy between late successional communities in Experiments I and II is the presence of a mucilaginous layer of *Navicula pelliculosa* on enriched substrata in the latter experiment (Figs. 3, 4). The negative additive response of *A. minutissima* to combined substratum and water enrichment (Table 4) is probably due to the location of this sessile taxon beneath the double canopy layers of (1) *N. pelliculosa* and (2) *Navicula* and *Nitzschia* spp. (Fig. 3). The biomass of upperstory biraphid taxa was two times as great on organic-P enriched substrata (Experiment II; Fig. 2B) as on inorganic-P enriched substrata (Experiment I; Fig. 2A). *Achnanthes minutissima* has been frequently observed as a dominant in nutrient-poor environments (Allanson 1973, Eminson 1978, Pringle 1985*a, b*). It is also known to have high oxygen requirements, and its abundance has been used as an indicator in assessing water reoxygenation after pollution (Cholnoky 1968). The continuous skin-like layer of *N. pelliculosa* cells undoubtedly inhibits turbulent diffusion of nutrients and oxygen into lower layers, allowing potentially toxic metabolic end products to accumulate. These factors may interfere with the metabolism of *A. minutissima.*

The abundance of *N. pelliculosa* on substrata enriched with β-glycerophosphate may be a function of response to elements other than phosphorus in this compound. *N. pelliculosa* can use glucose or glycerol as a carbon source for photoautotrophic growth in the absence of CO_2 (Jolley and Hellebust 1974). Also, the capsular material in which *N. pelliculosa* cells are enclosed is a polymer of glucoronic acid (Lewin 1955).

Specific taxa that were abundant on enriched substrata have been previously observed to be abundant in eutrophic environments: *Nitzschia palea* (Hustedt 1937, Jorgensen 1948, Cholnoky 1968), *N. linearis, N. cryptocephala,* and *N. tripunctata* (Jorgensen 1948). Members of the genus *Nitzschia* generally reach greatest abundance in eutrophic waters (Benecke 1900, Butcher 1946, 1947) and also show the most pronounced development of heterotrophic capabilities among diatoms (Hellebust and Lewin 1977).

The role of motility in determining periphyton community structure

Periphyton community physiognomy is closely tied to the ability of specific taxa to exploit resources from the substratum and overlying water. Motile biraphid diatoms not only dominated the upperstory flora of enriched substratum treatments after 2–3 wk (Figs. 2, 3), but also predominated on P-enriched substrata incubated in Carp Creek for up to 6 wk (Pringle and Bowers 1984). A similar predominance of motile taxa was observed on natural nutrient-rich microhabitats in the same stream. Sand-grain retreats of larval Chironomidae (Diptera) were covered with dense mats of motile diatom taxa (Pringle 1985*a*).

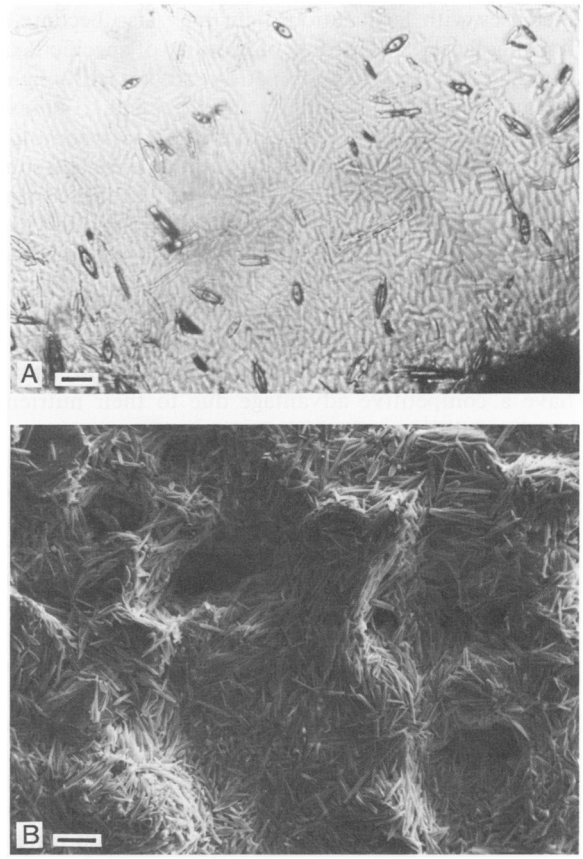

FIG. 4. Periphyton layers that have formed a substratum enriched with β-glycerophosphate and incubated in Carp Creek for 19 d. (A) Light micrograph of *Navicula pelliculosa* layer. Adjacent cells of *N. pelliculosa* form background "rice-grain" pattern. Cells are oblong, with thin almost translucent cell walls. Scale bar = 10 μm. (B) Scanning electron micrograph of overlying mat of *Navicula* and *Nitzschia* spp., where underlying sand grains and associated flora appear as mounds in the microtopography. Scale bar = 50 μm.

Hoagland et al. (1982) report that lake periphyton communities exhibited a microsuccessional trend from low to high physical stature. The motility of a diatom appears to confer a similar advantage in competing for surface area. Furthermore, overgrowth of the existing sessile community by motile taxa would appear to be more adaptive in conditions of high current than an increase in vertical stature, by providing less current resistance.

Overgrowth of underlying sessile taxa by motile diatoms complicates interpretations of succession based on taxon-specific nutrient uptake abilities. As biomass

increases with fertilization, light may also become a limiting factor. The degree of motility of specific taxa can therefore result in a competitive advantage for light in late stages of community development. (For example, after ≈3 wk of substratum colonization, motile diatoms form a thick upperstory mat on enriched substrata [Fig. 3].) Motile taxa, however, exhibited significantly greater rates of biovolume increase relative to nonmotile taxa early in colonization on enriched sand-agar substrata (Fig. 2A, B; 7–16 d, Experiment I; 7–15 d, Experiment II), before diatom mats were well formed and light limitation would presumably have occurred. This supports the hypothesis that motile taxa have a competitive advantage due to their nutrient uptake abilities.

The movement of flagellated phytoplankton is sufficient to overcome diffusion limitation (Pasciak and Gavis 1975) and sinking planktonic cells obtain a similar advantage by reducing the thickness of the depleted zone on the downward-directed cell surface (Smayda 1970). Motile diatom taxa have the ability to move freely among sediment particles, locating themselves in interstitial waters of the substratum that are often rich in nutrients and dissolved organic matter (Zicker et al. 1956, Abbot 1957, Senin 1970). Experimental results indicate that motile taxa can exploit nutrients in both the substratum and overlying waters, responding significantly to inorganic nutrients in both the substratum and water (Fig. 2A). Interpretation of results is further complicated by the fact that substratum and water nutrient sources are interchangeable and significant amounts of nutrients in overlying waters may become adsorbed to the sediment surface or to the periphyton mat itself.

Results indicate that motile taxa are much larger than sessile taxa, which may allow them to store large quantities of nutrients beyond their immediate physiological needs. Smaller sessile components of the diatom community that predominate on control substrata (e.g., *Achnanthes minutissima*) appear to be better adapted to exploit relatively low levels of dissolved nutrients within natural flowing waters, possibly by virtue of their high surface-to-volume ratio and growth habits (projecting into water flow). One valve surface of larger biraphid taxa such as *Navicula* spp. and *Nitzschia* spp. is in close contact with the underlying substratum, which would presumably facilitate extracellular enzymatic secretion and uptake of macromolecules. For instance, *Nitzschia filiformis* and *N. frustulum* secrete hydrolytic enzymes that digest agar, as evidenced by pit formation at sites of individual colonies (Lewin and Lewin 1960). Similar release of such enzymes by stalked, sessile taxa has not been recorded in the literature. Enzymatic dissolution of underlying substrata would presumably be detrimental to organisms with this mode of attachment. The stalked "turf" community would likewise appear to be poorly adapted for direct enzymatic uptake from sediments.

Periphyton substratum specificity in response to enrichment

Substratum specificity did not break down in response to enrichment, in contrast to the observations of Eminson and Moss (1980) for periphyton–macrophyte associations in a lake. In their study, however, nutrient levels were considerably higher (e.g., TP was 100–300 μg/L) than levels of enrichment in Carp Creek (e.g., TP, 20–25 μg/L).

Uniformity of surface texture has been frequently used as a prime criterion in the choice of artificial substrata (e.g., Lowe and Gale 1980). While uniformity of substratum surface texture between replicates is desirable because it minimizes sample variability, lack of texture within a given replicate may be an undesirable characteristic, depending on objectives of a given investigation. As this and previous studies have indicated, untextured substrata such as glass slides select against various algal taxa (Muntenau and Malay 1981). Surface heterogeneity within an artificial substratum habitat may, therefore, make it a more representative and effective sampling unit of the whole community. Significantly greater taxon richness and diversity on sand-agar substrata over glass slides agrees with the prediction that for a given level of resource richness, increases in surface heterogeneity should lead to increased species richness (Tilman 1982). The irregular surface topography of sand-agar substrata creates a greater variety of microhabitats and microcurrent regimes that may not only affect resource supply rates, algal immigration and emigration rates (Stevenson 1983), but also flow resistance, conferring an advantage to those taxa selected against on glass slides. While small monoraphid taxa such as *Cocconeis placentula* have been observed to withstand a shear stress of 10 Pa, epipelic taxa collected from silt were generally unable to withstand 1 Pa (Harper and Harper 1967).

Given that natural habitats are generally composed of a variety of substratum types and textures and that the response of specific diatom taxa to nutrient enrichment is not consistent among communities on different substratum types, bioassays of overall periphyton response to nutrient enrichment should optimally use a variety of artificial substrata that simulate various substratum types in the system. The magnitude of response of the epipelic algal community to enrichment of aquatic systems has been underestimated by the widespread use of glass slide substrata which support algal species associations that differ from epipelic community asssociations (Tippet 1970). In the present study, biraphid taxa accounted for >69% of the overall periphyton response on sand-agar substrata. If glass slides had been used alone to assess taxon response to a given nutrient, response of biraphid taxa may have been overlooked due to their low relative biovolume and the response of underlying sessile taxa overestimated due to their abundance and lack of overgrowth by motile taxa.

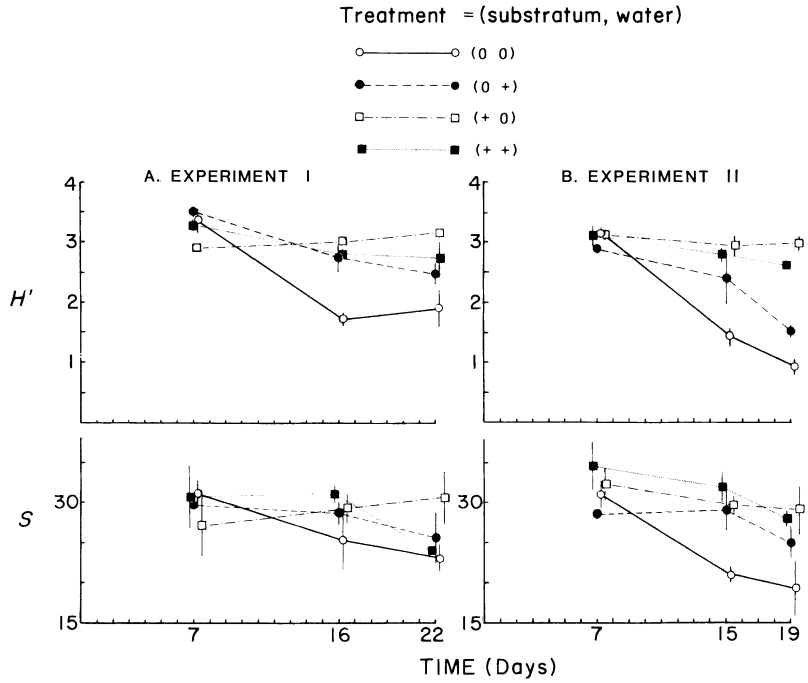

FIG. 5. Taxon diversity (H') and richness (S) on sand-agar substrata for (A) Experiment I (inorganic N, inorganic P) and (B) Experiment II (inorganic N, organic P). Data are means ± SD; $n = 3$ for each value. Characters in parentheses indicate whether nutrient enrichment was present (+) or absent (0) in the substratum (first character) or in the water (second character).

Periphyton taxon diversity

In aquatic and terrestrial habitats nutrient enrichment frequently results in decreased taxon diversity, with types of enrichment influencing which taxa become dominant (e.g., Beadle 1966, Russel-Hunter 1970, Schelske and Stoermer 1971). In experiments described here, however, competitive exclusion did not occur in response to nutrient treatments, as evidenced by greater diversity and taxon richness on all nutrient treatments relative to controls (Fig. 5). On first glance, these observations do not appear to be consistent with the well-substantiated prediction that taxon richness decreases with enrichment (Tilman 1982). However, Tilman's theory has an important qualifier that accommodates my results: in resource-poor habitats diversity is predicted to increase with resource richness through a narrow range. Enrichment experiments described here were conducted in nutrient-poor waters. Data points representing diatom species richness on fertilized treatments would thus fit into the ascending portion of the classic, humped, resource richness–species richness curve (Tilman 1982). Other studies have also documented this increase in taxon richness with resource richness through a narrow range (e.g., Beadle 1966, Holdridge et al. 1971, Ashton 1977).

Results support Tilman's (1982) prediction that the level of spatial heterogeneity (variance in resource availability between microhabitats) determines the maximum number of species that can coexist. Taxon richness and diversity is greatest on enriched substrata exposed to low ambient nutrient levels (Fig. 5). When low ambient nutrient levels are supplemented with N and P, nutrient heterogeneity between enriched substrata and water decreases. Taxon richness and diversity exhibited a corresponding decrease (Fig. 5). Highest taxon diversity was also found to occur on natural nutrient-diffusing substrata (retreats of larval chironomids) in Carp Creek, relative to more nutritionally inert substrata (Pringle 1985*a*). It has been similarly suggested that increases in amounts of dissolved organic substances derived from phytoplankton (Fogg 1965) and macrophytes (Eminson 1978) could provide more metabolic substrates for the phytoplankters themselves and attached epiphytes, respectively. This would create more niches without altering the dominance of any particular species, thereby increasing the diversity of the population.

This study provides experimental evidence that taxon diversity and richness are affected by spatial heterogeneity of nutrient resources in the substratum and water. Results support the hypothesis that high diversity is maintained in nutrient-poor systems by the natural heterogeneity of nutrients occurring in these systems relative to nutrient-rich environments. In nutrient-rich systems, the natural spatial heterogeneity of nutrients (e.g., patches resulting from algal extracellular release, invertebrate excreta, sediment and macrophyte release, etc.) is masked by the overriding effects of high nutrient levels in the water. The fact that epipelic communities are located at the interface of two, frequently different chemical environments (water and substra-

tum) may explain Hutchinson's observation that the species diversity of algae is frequently greater on the sediments than in the open water of lakes and ponds (Hutchinson 1975).

In conclusion, there is a strong relationship between the composition of lotic periphyton communities and the spatial heterogeneity of nutrient resources. The source of nutrient (substratum or water), form of nutrient (organic or inorganic), and substratum type can affect periphyton taxon specific response to nutrients. Periphyton community physiognomy and interactions between taxa are important, interrelating factors regulating algal response to nutrients from different sources.

Acknowledgments

This paper is dedicated to the memory of Peter Kilham. I thank J. Affolter, F. Hooper, P. Kilham, J. Bowers, and F. Triska for their helpful comments on the manuscript and both E. Kimanani and A. Jensen for advice regarding statistical analyses. Thanks are also extended to S. Fisher and two anonymous reviewers for their comments and editorial advice. J. Lufkin is gratefully acknowledged for her excellent line drawing. This study is part of a Ph.D. dissertation submitted to the University of Michigan in 1985. Research was supported by a National Science Foundation Grant BSR-87-17746 to C. M. Pringle, a Rackham Dissertation Improvement Grant, and an ARCO grant for field research.

Literature Cited

Abbot, W. 1957. Unusual phosphorus source for plankton algae. Ecology **38**:152.

Allanson, B. R. 1973. The fine structure of the periphyton of *Chara* sp. and *Potamogeton natans* from Wytham Pond, Oxford and its significance to the macrophyte periphyton model of R. G. Wetzel and H. L. Allen. Freshwater Biology **2**:535–542.

American Public Health Association (APHA). 1980. Standard methods for the examination of water and wastewater. 15th edition. American Public Health Association, Washington, D.C., USA.

Armstrong, F. A. J., C. R. Sterns, and J. D. H. Strickland. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon autoanalyzer and associated equipment. Deep Sea Research **14**:381–389.

Ashton, P. S. 1977. A contribution of rainforest research to evolutionary theory. Annals of the Missouri Botanical Garden **64**:694–705.

Beadle, N. C. W. 1966. Soil phosphate and its role in molding segments of the Australian flora and vegetation, with special reference to xeromorphy and sclerophylly. Ecology **47**:992–1007.

Benecke, W. 1900. Über farblose Diatomeen der Kieler Forde. Jahrbucher für Wissenschaftlichen Botanik **35**:535–547.

Butcher, R. W. 1946. The biological detection of pollution. Journal of the Institute of Sewage Purification **2**:92–97.

———. 1947. Studies on the ecology of rivers. VII. The algae of organically enriched waters. Journal of Ecology **35**: 186–191.

Cholnoky, B. J. 1968. Die Okologie der Diatomeen in Binnengwassern. J. Cramer, Lehre, Germany.

Elwood, J. W., J. D. Newbold, R. V. O'Neill, R. W. Stark, and P. T. Singley. 1981*a*. The role of microbes associated with organic and inorganic substrates in phosphorus spiralling in a woodland stream. Verhandlungen Internationale Vereinigung für theoretische und angewandte Limnologie **21**:850–856.

Elwood, J. W., J. D. Newbold, A. F. Trimble, and R. W. Stark. 1981*b*. The limiting role of phosphorus in a woodland stream ecosystem: effect of P enrichment on leaf decomposition and primary producers. Ecology **62**:146–158.

Eminson, D. F. 1978. A comparison of diatom epiphytes, their diversity and density, attached to *Myriopyllum spicatum* L. in Norfolk dykes and broads. British Phycological Journal **13**:57–64.

Eminson, D., and B. Moss. 1980. The composition and ecology of periphyton communities in freshwater. I. The influence of host type and external environment on community composition. British Phycological Journal **15**:429–446.

Fitzgerald, G. P., and T. C. Nelson. 1966. Extractive and enzymatic analyses for limiting or surplus phosphorus in algae. Journal of Phycology **2**:32–37.

Fogg, G. E. 1965. Algal cultures and phytoplankton ecology. Athlone, London, England.

Grimm, N. B. 1986. Nitrogen limitation in a Sonoran desert stream. Journal of the North American Benthological Society **5**:2–15.

Gruendling, G. K. 1971. Ecology of the epipelic algal communities in Marion Lake, British Columbia. Journal of Phycology **7**:239–249.

Hamilton, P. B., and H. C. Duthie. 1984. Periphyton colonization of rock surfaces in a boreal forest stream studied by scanning electron microscopy and track autoradiography. Journal of Phycology **20**:525–532.

Harper, M. A., and J. F. Harper. 1967. Measurements of diatom adhesion and their relationship with movement. British Phycological Bulletin **3**:195–207.

Healey, F. P. 1973. Characteristics of phosphorus deficiency in *Anabaena*. Journal of Phycology **9**:383–394.

Hellebust, J. A., and J. Lewin. 1977. Heterotrophic nutrition. Pages 169–197 *in* D. Werner, editor. The biology of diatoms. Blackwell Scientific, London, England.

Hoagland, K. D. 1983. Short-term standing crop and diversity of periphytic diatoms in a eutrophic reservoir. Journal of Phycology **19**:30–38.

Hoagland, K. D., S. C. Roemer, and J. R. Rosowski. 1982. Colonization and community structure of two periphyton assemblages with emphasis on the diatoms (Bacillariophyceae). American Journal of Botany **69**:188–213.

Hohn, M. H., and J. Hellerman. 1963. The taxonomy and structure of diatom populations from three eastern North American rivers using three sampling methods. Transactions of the American Microscopical Society **82**:250–329.

Holdridge, L., W. Grenke, W. Hatheway, T. Liang, and J. Tosi, Jr. 1971. Forest environments in tropical life zones: a pilot study. Pergamon, London, England.

Holm-Hansen, O., C. J. Lorenzen, R. W. Holmes, and J. D. H. Strickland. 1965. Fluorometric determination of chlorophyll. Journal du Conseil International pour l'Exploration de la Mer **30**:3.

Hudon, C., and E. Bourget. 1981. Initial colonization of artificial substrates: community development and structure studied by scanning electron microscopy. Canadian Journal of Fisheries and Aquatic Sciences **38**:1371–1384.

Hudon, C., and E. Bourget. 1983. The effects of light on the vertical structure of epibenthic diatom communities. Botanica Marina **26**:317–330.

Hudon, C., and P. Legendre. 1987. The ecological implications of growth forms in epibenthic diatoms. Journal of Phycology **23**:434–441.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs **54**: 187–211.

Hustedt, F. 1937. Systematische und Okologische Unter-

suchungen über die Diatomeenflora von Java, Bali und Sumatra. Archiv für Hydrobiologie, Supplement (Stuttgart) **15**:131–177.

Hutchinson, G. E. 1975. A treatise on limnoloy. Volume III. Limnological botany. John Wiley & Sons, New York, New York, USA.

Jolley, E. T., and J. A. Hellebust. 1974. Preliminary studies on the nutrition of *Navicula pelliculosa* (Breb.) Hilse, and an associated bacterium, *Flavobacterium* sp. Journal of Phycology **10** (Supplement):7.

Jorgensen, E. G. 1948. Diatom communities in some Danish lakes and ponds. Kongelige Danske Videnskabernes Selskab Biologiske Skrifter (Copenhagen) **5**:1–140.

Korte, V. L., and D. W. Blinn. 1983. Diatom colonization on artificial substrata in pool and riffle zones studied by light and scanning electron microscopy. Journal of Phycology **19**:332–341.

Koshland, D. E. 1965. Mechanisms of transfer enzymes. Pages 305–346 *in* P. D. Boyer, H. Lardy, and K. Myrback, editors. The enzymes. Academic Press, New York, New York, USA.

Kuenzler, E. J., and J. P. Perras. 1965. Phosphatases of marine algae. Biological Bulletin **128**:271–285.

Lewin, J. C. 1955. The capsule of the diatom *Navicula pelliculosa*. Journal of General Microbiology **13**:162–169.

Lewin, J. C., and R. A. Lewin. 1960. Auxotrophy and heterotrophy in marine littoral diatoms. Canadian Journal of Microbiology **6**:127–134.

Lowe, R. L., and W. F. Gale. 1980. Monitoring river periphyton with artificial benthic substrates. Hydrobiologia **69**:235–244.

Luttenton, M. R., and R. G. Rada. 1986. Effects of disturbance on epiphytic community architecture. Journal of Phycology **22**:320–326.

Muntenau, N., and E. J. Malay. 1981. The effect of current on the distribution of diatoms settling on submerged glass slides. Hydrobiologia **78**:278–282.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta **27**:30.

Pasciak, W. J., and J. Gavis. 1975. Transport limited nutrient uptake rates in *Ditylum brightwelli*. Limnology and Oceanography **20**:604–617.

Patni, N. J., S. W. Dhawale, and S. Aaronson. 1977. Extracellular phosphatases of *Chlamydomonas reinhardi* and their regulation. Journal of Bacteriology **130**:205–211.

Patrick, R., and M. H. Hohn. 1956. The diatometer—a method for indicating the conditon of aquatic life. Proceedings of the American Petrology Institute, Section 3, **36**: 332–338.

Patrick, R., M. H. Hohn, and J. H. Wallace. 1954. A new method for determining the pattern of the diatom flora. Academy of Natural Sciences of Philadelphia, Notulae Naturae **259**:1–12.

Peterson, B. J., J. E. Hobbie, T. L. Corliss, and K. Kriet. 1983. A continuous flow periphyton bioassay: tests of nutrient limitation in a tundra stream. Limnology and Oceanography **28**:583–591.

Pielou, E. C. 1966. The measurement of diversity of different types of biological collections. Journal of Theoretical Biology **13**:131–144.

Pringle, C. M. 1979. The herbivorous feeding behavior of *Baetis* with special reference to the effects of chironomid tube-building activities on the diatom flora. Thesis. University of Michigan, Ann Arbor, Michigan, USA.

———. 1985*a*. Effects of chironomid (Insecta: Diptera) tube-building activities on stream diatom communities. Journal of Phycology **21**:185–194.

———. 1985*b*. Nutrient heterogeneity and the maintenance of species diversity: periphyton response to substratum and water nutrient enrichment in a nutrient-poor stream. Dissertation. University of Michigan, Ann Arbor, Michigan, USA.

———. 1987. Effects of water and substratum nutrient supplies on lotic periphyton growth: an integrated bioassay. Canadian Journal of Fisheries and Aquatic Sciences **44**: 619–629.

Pringle, C. M., and J. A. Bowers. 1984. An *in situ* substratum fertilization technique: diatom colonization on nutrient enriched sand substrata. Canadian Journal of Fisheries and Aquatic Sciences **41**:1247–1251.

Pringle, C. M., P. Paaby-Hansen, P. D. Vaux, and C. R. Goldman. 1986. *In situ* nutrient assays of periphyton growth in a Lowland Costa Rican stream. Hydrobiologia **134**:207–213.

Redfield, A. C., B. H. Ketchum, and F. A. Richards. 1963. The influence of organisms on the composition of seawater. Pages 26–77 *in* M. N. Hill, editor. The Sea. Volume 2. Wiley Interscience, New York, New York, USA.

Rhee, G. Y. 1972. Competition between an alga and an aquatic bacterium for phosphate. Limnology and Oceanography **17**:505–514.

———. 1978. Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition and nitrate uptake. Limnology and Oceanography **23**:10–25.

Russel-Hunter, W. 1970. Aquatic productivity. Macmillan, New York, New York, USA.

Schelske, C. L., and E. F. Stoermer. 1971. Eutrophication, silica depletion, and predicted changes in algal quality in Lake Michigan. Science **173**:423–424.

Searle, S. R. 1971. Linear models. John Wiley & Sons, New York, New York, USA.

Senin, Y. M. 1970. Phosphorus in bottom sediments of the South West African Shelf. Litologiia i Poleznye Iskopaemye **1**:11–26.

Smayda, T. J. 1970. The suspension and sinking of phytoplankton in the sea. Oceanography and Marine Biology Annual Review **8**:353–414.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. Second edition. W. H. Freeman, San Francisco, California, USA.

Steinman, A. D., and C. D. McIntire. 1986. Effects of current velocity and light energy on the structure of periphyton assemblages in laboratory streams. Journal of Phycology **22**:352–361.

Steinman, A. D., C. D. McIntire, S. V. Gregory, G. A. Lamberti, and L. R. Ashkenas. 1987. Effects of herbivore type and density on taxonomic structure and physiognomy of algal assemblages in laboratory streams. Journal of the North American Benthological Society **6**:175–188.

Stevenson, R. J. 1983. Effects of current and conditions simulating autogenically changing microhabitats on benthic diatom immigration. Ecology **64**:1514–1524.

Stockner, J. G., and K. R. Shortreed. 1978. Enhancement of autotrophic production by nutrient addition in a coastal rainforest stream on Vancouver Island. Journal of the Fisheries Research Board of Canada **35**:28–34.

Tilman, D. 1982. Resource competition and community structure. Monographs in Population Biology **17**.

Tippet, R. 1970. Artificial surfaces as a method of studying populations of benthic micro-algae in freshwater. British Phycological Journal **5**:187–199.

Triska, F. J., V. C. Kennedy, R. J. Avanzino, and B. N. Reilly. 1983. Effect of simulated canopy cover on regulation of nitrate uptake and primary production by natural periphyton assemblages. Pages 129–159 *in* T. D. Fontaine and S. M. Bartell, editors. Dynamics of lotic ecosystems. Ann Arbor Science Publishers, Ann Arbor, Michigan, USA.

Wetzel, R. G. 1981. Longterm dissolved and particulate

alkaline phosphatase activity in a hardwater lake in relation to lake stability and phosphorus enrichments. Internationale Vereinigung für theoretische und angewandte Limnologie, Verhandlungen **21**:369–381.

Whittaker, R. H. 1961. Experiments with radiophosphorus tracer in aquarium microcosms. Ecological Monographs **31**: 157–188.

Zicker, E. L., K. C. Berger, and A. D. Hasler. 1956. Phosphorus release from bog lake muds. Limnology and Oceanography **1**:296–303.