Oecologia (1991) 86:287–291

Oecologia

Grazer control of nutrient availability in the periphyton

Paul V. McCormick* and R. Jan Stevenson

Department of Biology, University of Louisville, Louisville, KY 40292, USA

Received April 3, 1990 / Accepted in revised form November 6, 1990

Summary. Benthic algal assemblages are regulated by both abiotic (e.g., nutrient) and biotic (e.g., grazing) constraints. The objective of this study was to determine how changes in these two factors affected the structure of an algal assemblage in an ephemeral stream. Coverslips were incubated for 21 days in enclosures containing one of three nutrient environments (ambient, phosphorus-enriched, or phosphorus and nitrogen enriched) and one of four densities of the snail *Goniobasis* (0, 40, 80, or 120 snails/m^2) and examined directly to enumerate the algal assemblage. The effect of grazing on algal biomass was dependent on the nutrient environment. An overstory of diatoms was susceptible to removal by grazing and was not strongly affected by nutrient enrichment. An understory of *Stigeoclonium* was more resistant to grazing and responded strongly to nutrient enrichment only in the presence of grazers. Snail grazers may mediate nutrient availability to the understory indirectly by removing overlying cells or by direct excretion of nutrients. Multiple interactions occur between benthic herbivores and algae, and, as shown here, some of them are positive and involve modifications of the nutrient environment.

Key words: Diatoms – *Goniobasis* – Herbivory – Nutrient availability – Periphyton

The structure of benthic algal assemblages is affected by both endogenous and exogenous factors. Recent studies have shown interactive effects between some of these factors (e.g., grazing and resource availability). Of course, grazing decreases benthic algal accumulation rates (e.g., Sumner and McIntire 1982; Cuker 1983; Lamberti and Resh 1983; McAuliffe 1984; Hart 1985; Steinman et al. 1987, 1989) and nutrient and light resources stimulate accumulation (e.g., Stockner and Shortreed 1978; Bothwell 1985; Steinman and McIntire 1987; Hill and Knight 1988). The additional primary production caused by resource supplements (e.g., light) can increase secondary production (Lamberti et al. 1989). However, recent evidence suggests grazers positively affect the supply of resources (e.g., nutrients) to the grazer-resistant algal understory (McCormick and Stevenson 1989). Grazers may increase nutrient supply indirectly by removing the periphyton overstory and facilitating diffusion from water column to the understory. In addition, grazers excrete nitrogen (Friedl 1974; Grimm 1988) and may increase nutrient supply directly.

The objective of this study was to test for independent and interactive effects of invertebrate grazing and nutrient availability. A previous study (McCormick and Stevenson 1989) indicated that the extent of nutrient (phosphorus) limitation affected the response of benthic algal assemblages to invertebrate grazing. This question was investigated further in the present study by adding this limiting resource alone and in combination with a second resource (nitrogen) which may be limiting to some algal populations. Sampling and counting methods enabled direct observation of algal assemblages and physiognomy to determine the mechanisms of grazing effects.

Methods

Study site and preliminary observations

Wilon Creek is a third order stream in Bernheim Forest, a private wildlife sanctuary located approximately 60 km south of Louisville, KY, USA. Although the creek is partially shaded, it supports abundant algal growth for much of the year. Like many small streams in the region, Wilson Creek is ephemeral; surface flow often ceases by early summer each year and the channel becomes fragmented into a series of isolated pools. Pooling temporarily isolates and concentrates invertebrate and vertebrate consumers. One of the dominant invertebrates present in these summer pools and used in this study is the gastropod *Goniobasis curreyana*. Benthic algal

* *Present address and address for offprint requests:* University Center for Environmental and Hazardous Materials Studies, 1020 Derring Hall, VPI & SU, Blacksburg, VA 24061, USA

assemblages in pools containing high densities of this herbivore are dominated by basal holdfast cells of the chlorophyte *Stigeoclonium*. These cells form a patchy "lawn" of prostrate algae on the substrate.

Most of the summer pools in Wilson Creek are shallow and persist in bedrock basins which receive little or no subsurface flow. Consequently, physico-chemical conditions are altered drastically as summer pooling persists compared to conditions during flow. In particular, level of macronutrients (e.g., phosphorus and nitrogen) can become limiting as the summer progresses. Algal assemblages in pools containing large herbivore populations tend to be phosphorus limited (McCormick and Stevenson 1989), while those with few or no herbivores tend toward nitrogen limitation (McCormick 1990; Stevenson unpublished).

The pool used in the present experiment had a surface area of 30 m^2 and a maximum depth of 25 cm. The dominant grazer, *Goniobasis curreyana*, was present at a density of 115 individuals/m^2 during experimentation, as determined by averaging the number of snails present in 20 randomly selected 78.5 cm^2 plots. As with the benthic algae, snails tended to be patchily distributed and many plots had no individuals present. The benthic algal assemblage in this pool was previously found to be most limited by phosphorus (McCormick and Stevenson 1989).

Experimental design

Because of differences in surface area, depth, shading and other physical characteristics among pools in Wilson Creek, effects of herbivory by *Goniobasis* on the benthic algal assemblage were determined using replicate enclosures placed in the pool described above. While this approach limited generalizations which could be made among pools, it satisfied the objectives of this study, which were to explore the ways in which grazing and nutrients affected a benthic algal community.

Experimental enclosures were 200 ml acid-rinsed glass culture dishes. The open top of each dish was covered with nylon netting (2 mm mesh) to allow free passage of water and algal cells between the enclosure and the pool environment while excluding snails present in the pool. Glass coverslips (484 mm^2), affixed to the inside walls of enclosures with a small amount of petroleum jelly, were used as collectable surfaces for algal growth.

Forty-eight enclosures were used during September and October, 1987 to determine how grazing and local nutrient availability affected benthic algae. Each of twelve enclosures received either 0, 1, 2, or 3 snails, which ranged in size from approximately 15–20 mm. These densities corresponded to 0, 40, 80, and 120 snails/m^2, respectively.

Enclosures containing each snail density were enriched with either no nutrients, one of two types of phosphorus salts, or both nitrogen and phosphorus salts (three replicates/treatment). Nutrient enrichment was achieved by adding a clay saucer (10 cm diameter) containing a mixture of 4% agar and one of the following nutrient additions: 1) no nutrient salts; 2) 0.25 M KH_2PO_4; 3) 0.25 M NaH_2PO_4; 4) 0.25 M NaH_2PO_4 and 0.25 M KNO_3. Two types of phosphorus treatments were used to determine if the addition of the cations K^+ or Na^+, which were not of interest but were associated with the nutrient anions of interest, influenced algal growth responses (see Fairchild et al. 1985). The combined nitrogen-phosphorus enrichment was used to determine the importance of secondary nutrient limitation by a second macronutrient (nitrogen) when the primary limiting nutrient (phosphorus) was being supplied.

Enclosures were placed approximately 30 cm apart on a flat area of substrate in the pool. Enclosures of each treatments were randomly assigned to spaces within this area to insure that there was no structured dependency in the experimental design.

Algal assemblages in the enclosures were sampled after 21 days incubation by: 1) carefully lifting individual enclosures out of the stream pool and onto a level sampling platform; 2) carefully removing the two coverslips from each enclosure with forceps; 3) placing the coverslips, exposed side down, onto a 75 mm × 50 mm glass slide containing a thin coating of 300% Taft's syrup medium (see Stevenson 1984). These mounts were allowed to dry in the laboratory for approximately two weeks at which time the upper surface of each was cleaned with concentrated HNO_3 and 95% ETOH to permit microscopical examination. Abundances of dominant species were determined at 1000 × using oil immersion. Algal cell densities were enumerated in randomly chosen microscope fields until a minimum of 500 algal cells were counted on each coverslip from an enclosure.

The mortality of enclosed snails was determined concurrently with sampling of the algal assemblage. Water-column nutrient concentrations in the enclosures were determined on day 21 by filtering the water drained from each enclosure through a 0.45 µm membrane filter into acid washed sampling vials and measuring concentrations of nitrate and soluble reactive phosphorus using standard methods (APHA et al. 1985).

Data analysis

All statistical analyses were performed using the Statistical Analysis System (SAS 1985). Data were natural log transformed where necessary to increase homogeneity of variance among treatments (Sokal and Rohlf 1981).

Differences in water column nutrient concentrations, total algal density, total algal biovolume, and the density of dominant species among nutrient and grazing treatments were detected using a two-way ANOVA followed by protected LSD's (Lentner and Bishop 1986) when ANOVA results were statistically significant.

Results

Efficacy of grazing and nutrient manipulations

All snails were alive in enclosures at the end of the experiment. Most snails were observed on the glass sides of the enclosures, although occassionally individuals were observed either directly on the nutrient diffusing substrate or on the netting placed over the glass enclosure. Preference for the glass sides of the enclosure was likely due to the visually greater amounts of periphyton materil on this surface; as in many horizontal parts of the pool bottom, the bottom of the enclosures was covered with silt which tended to inhibit periphyton growth.

Water in enclosures containing phosphorus-diffusing substrates had significantly higher levels of soluble reactive phosphorus than that in unenriched enclosures ($p < 0.05$, protected LSD, Fig. 1). Water in enclosures containing nitrogen-diffusing substrates had significantly higher levels of nitrate than that in unenriched enclosures ($p < 0.05$, protected LSD).

Response of algal biomass

In the absence of nutrient enrichment, there was a decrease in total cell density of 40 snails/m^2 and biovolume at 80 snails/m^2 compared with ungrazed enclosures ($p < 0.05$, protected LSD, Fig. 2a and b). Increases in snail density above 80 snails/m^2 elicited no additional response ($p > 0.05$, protected LSD).

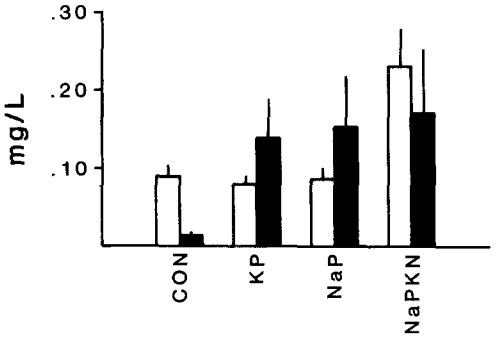

Fig. 1. Water column concentrations of nitrate (*open bars*) and soluble reactive phosphate (*closed bars*) inside ungrazed enclosures of each nutrient treatment on day 21

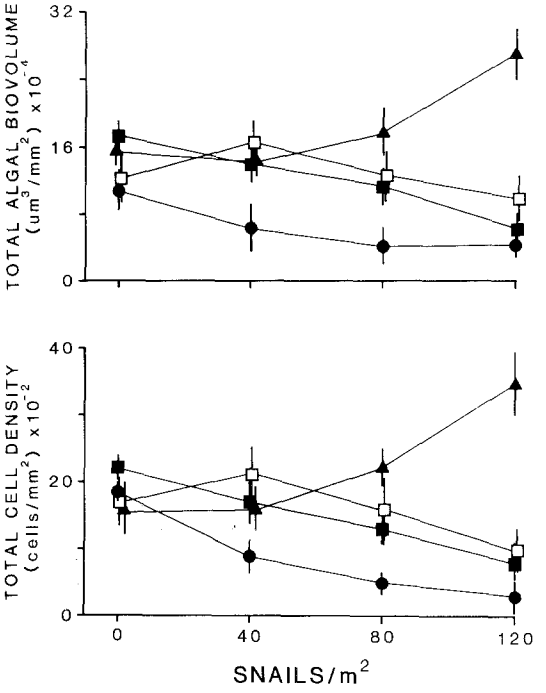

Fig. 2. Total algal biovolume and total algal cell density in enclosures enriched with 1) no nutrient (*closed circles*), 2) potassium phosphate salt (*closed squares*), 3) sodium phosphate salt (*open squares*), 4) sodium phosphate and potassium nitrate salts (*closed triangles*) and exposed to different densities of *Goniobasis*. Points are means of three replicates; error bars are standard errors of the means

In the absence of grazing there was no significant effect of either type of phosphorus enrichment compared to the unenriched environment ($p > 0.05$, protected LSD). At intermediate snail densities (40–80 snails/m^2), phosphorus enrichment resulted in a two-fold increase in algal density and biovolume compared to controls ($p < 0.05$, protected LSD). Phosphorus enrichment had no significant effect on biomass parameters at high (120 snails/m^2) snail densities ($p > 0.05$, protected LSD).

The response of biomass parameters to combined enrichment with phosphorus and nitrogen was markedly different from that seen in other nutrient environments. As with phosphorus enrichment alone, combined enrichment did not significantly increase biomass parameters compared to unenriched conditions in the absence of grazing. However, both total algal cell densities and total algal biovolume increased steadily with increased grazing pressure in enclosures with combined enrichment. Algal density and biovolume were higher at the highest snail density with combined nitrogen and phosphorus enrichment than in any other treatment ($p < 0.05$, protected LSD).

Response of algal populations

Five taxa accounted for over 95% of the total density and biovolume of algal cells in all treatments, including four diatom species (*Achnanthes minutissima, Cyclotella meneghiniana, C. stelligera,* and *Nitzschia acicularis*) and the filamentous green algae, *Stigeoclonium*. *Stigeoclonium* was present mainly as clusters of prostrate basal holdfast cells, although short filaments were occasionally encountered growing fom the holdfast cells. The four diatom species grew in a thin mucilaginous layer over the *Stigeoclonium* cells.

The abundance of all four diatom species decreased in response to increased grazing in all four nutrient environments (Fig. 3). Both species of *Cyclotella* were extremely susceptible to grazing, although combined enrichment of phosphorus and nitrogen minimized the effect of low (40 snails/m^2) snail densities on *C. meneghiniana* compared to other nutrient treatments ($p < 0.05$, protected LSD). Enrichment with phosphorus enhanced the abundance of *Nitzschia acicularis* compared to unenriched enclosures ($p < 0.05$, protected LSD), but had little effect on its response to grazing. *Achnanthes minutissima* was somewhat more resistant to grazing than other dominant diatoms, although the density of this species was still significantly reduced at high (120 snails/m^2) snail densities compared to ungrazed enclosures ($p < 0.05$, protected LSD).

The four diatom species and their associated mucilage comprised an algal overstory over *Stigeoclonium* basal cells. This overstory (the sum of the cell densities of the four species) decreased with increases in grazing from 0 to 40 snails/m^2 in all nutrient environments ($p < 0.05$, protected LSD), but did not decrease further with increases from 40 to 120 snails/m^2 ($p > 0.05$, protected LSD). This response essentially mirrored that of *Cyclotella stelligera*, which comprised the bulk of the diatom overstory.

The chlorophyte *Stigeoclonium* was resistant to grazing in the absence of enrichment ($p > 0.05$, protected LSD). Enrichment with phosphorus alone increased the density of this species compared to unenriched conditions only at intermediate (80 snails/m^2) snail densities ($p < 0.05$, protected LSD). Under conditions of combined enrichment, cell densities of *Stigeoclonium* increased steadily with increased grazing pressure. At the highest snail density, this species comprised almost all of the algal biovolume on the substrate and was present at higher densities than in any other treatment ($p < 0.05$, protected LSD).

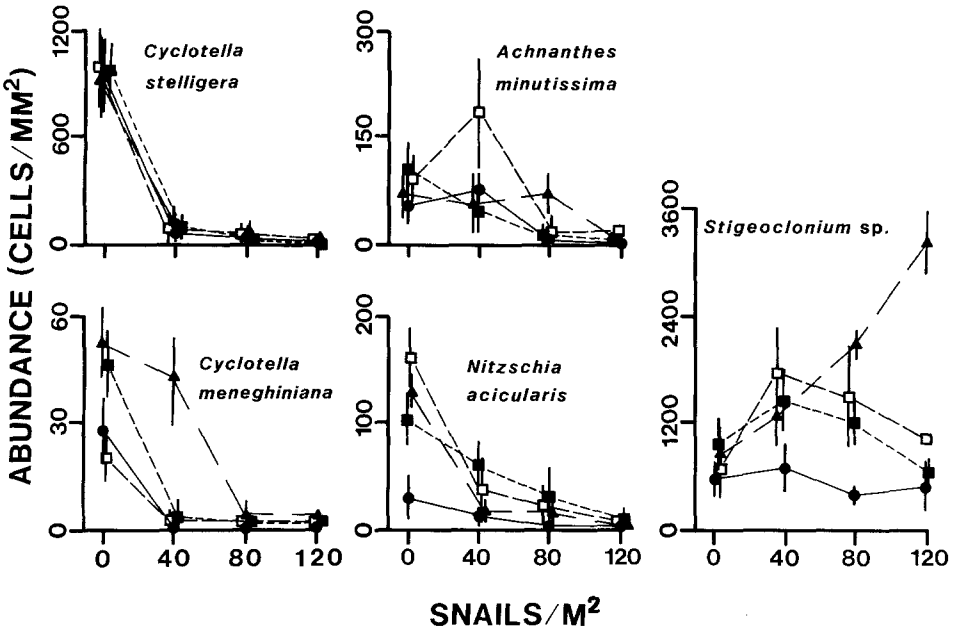

Fig. 3. Density of dominant algal species in enclosures enriched with different nutrients and exposed to different densities of *Goniobasis*. See Fig. 2 for legend

Discussion

Grazing usually causes a reduction in total algal biomass, but individual algal populations can exhibit a positive response to increases in grazing intensity (McCormick and Stevenson 1989; McCormick 1990; Steinman et al. 1989) as observed in this study. Indeed, positive population responses can outweigh negative population responses in certain nutrient environments, and algal biovolume can increase with increased grazing.

Interspecific differences in resistance to grazing among algal species have been attributed to differing cell sizes (Sumner and McIntire 1982), growth forms (Patrick 1970; Kesler 1981; Sumner and McIntire 1982) and/or growth potentials (Sumner and McIntire 1982). In the present study, negative responses to grazing may have been strongly influenced by growth form. All dominant species, except for *Stigeoclonium*, were loosely attached to the substrate in a thin mucilaginous layer and were, therefore, susceptible to removal by *Goniobasis*. Basal cells of *Stigeoclonium* were extremely resistant to removal, and comprised the bulk of *Stigeoclonium* biomass, especially in heavily grazed treatments.

Accumulation rates of most dominant diatom species were not stimulated by phosphorus and nitrogen enrichment in the absence of grazing. A variety of factors other than nitrogen and phosphorus may have limited growth of overstory diatoms, such as silica (Carrick et al. 1988), micronutrients (Pringle et al. 1986), or inorganic carbon (Fairchild and Sherman 1989). Growth of one overstory species, *Nitzschia acicularis*, was clearly limited by phosphorus, although addition of this nutrient did not affect its abundance in the presence of grazers. Only the growth of the understory species, *Stigeoclonium*, was stimulated by nutrient enrichment when grazers were present.

The pattern of response of *Stigeoclonium* to nutrient enrichment and grazing indicates that grazers have a strong effect on the nutrient response of understory species in benthic algal assemblages. In the absence of snail grazing, *Stigeoclonium* exhibited no significant response to nutrient enrichment. In the presence of grazers, the response of *Stigeoclonium* to combined nutrient enrichment was proportional to the density of snails. Two mechanisms are hypothesized for the observed positive effects of grazers on the most grazer-resistant algae: 1) grazers themselves may excrete nutrients and directly increase nutrient supply (Friedl 1974; Grimm 1988); 2) grazers may indirectly increase nutrient supply to understory algae by removing overstory diatoms.

Transport rates of nutrients into periphyton mats can be slowed by even relatively low densities of overstory periphyton (Riber and Wetzel 1987; Stevenson and Glover 1990). It is possible, therefore, that removal of the overstory diatom community by *Goniobasis* resulted in increased penetration of nutrients to *Stigeoclonium* cells underneath. Although grazing may also increase the availability of other resources (e.g., light) in a similar manner, the primary effect here appeared to be on the availability of the amended nutrients, since *Stigeoclonium* did not respond to grazing in the absence of nutrient enrichment, even though overstory diatom density was still reduced by grazing. This indirect effect of grazers on nutrient availability would be expected to be maximized at moderate (e.g., 40 snails/m^2) snail densities, where overstory biomass reached a minimum. The increase in *Stigeoclonium* density at higher (e.g., 80 to 120 snails/m^2) grazer densities in nutrient-enriched conditions suggests that direct effects of nutrient excretion by *Goniobasis* may also be important, since overstory diatom densities had been reduced to a minimum at 40 snails/m^2, and, thus, removal of more overstory diatoms by the grazer did not appear to be a factor.

The positive responses of algae to grazing observed in this experiment may be dependent on several abiotic

and biotic factors. Obviously, a species must be relatively resistant to consumption in order for the population to benefit from changes in the nutrient environment caused by grazing. Since grazer-resistant cells tend to be those that exhibit a prostrate growth form, high rates of growth by these species may result in space limitations, which might become more important than nutrient availability in determining algal growth responses. While space did not appear to limit *Stigeoclonium* growth in Wilson Creek pools, where substantial amounts of the epilithic substrate had no visible algal growth, this factor may be increasingly important in high-resource environments where algal growth rates can be maximized. Finally, removal of overlying material by grazers may be most efficient in erosional habitats (e.g., riffles) or on vertical surfaces, such as those used in the present study; in depositional habitats within the pool used here (e.g., the deepest sections), a fine layer of silt up to several cm thick was retained despite noticeable snail activity.

The effect of grazers on benthic algae is usually considered in terms of direct consumptive (i.e., negative) effects. Grazers apparently affect competitive interactions among benthic algae by differentially reducing overstory abundances, thereby increasing the supply of nutrients, and possibly other resources, to the understory. Grazers also may increase nutrient supply directly by excretion, and our data suggest that those nutrients may stimulate growth of grazer resistant species. Indeed, if nutrient supply is adequate and a species is present that is highly resistant to grazing, as is *Stigeoclonium*, grazing can increase benthic algal standing crop if reproduction exceeds grazing rates.

Acknowledgements. This research was supported by a research grant from Sigma Xi and a doctoral research fellowship from the University of Louisville to the first author. F. Stuart Chapin, III and an anonymous reviewer provided several helpful comments on the manuscript. Mr. Charles McClure, III and the staff of the Bernheim Forest Foundation provided the study location and logistical support.

References

American Public Health Association, American Water Works Association, and Water Pollution Control Federation (1985) Standard methods for the examination of water and wastewater, 16th ed. Washington, D.C.

Bothwell ML (1985) Phosphorus limitation of lotic periphyton growth rates: an intersite comparison using continuous flow troughs (Thompson River System, British Columbia). Limnol Oceanogr 30:527–542

Carrick HJ, Lowe RL, Rotenberry JT (1988) Guilds of benthic algae along nutrient gradients: Relationships to algal community diversity. J N Am Benthol Soc 7:117–128

Cuker BE (1983) Grazing and nutrient interactions in controlling the activity and composition of the epilithic algal community of an arctic lake. Limnol Oceanogr 28:133–141

Fairchild GW, Lowe RL, Richardson WB (1985) Algal periphyton growth on nutrient-diffusing substrates: an in situ bioassay. Ecology 66:465–472

Fairchild GW, Sherman JW (1989) Nutrient control of periphyton growth in softwater lakes. Bull N Am Benthol Soc 6 (1):121

Friedl FE (1974) Nitrogen excretion by the freshwater snail *Lymnaea stagnalis jugularis* Say. Comp Biochem Physiol 49:617–622

Grimm NB (1988) Role of macroinvertebrates in nitrogen dynamics of a desert stream. Ecology 69:1884–1893

Hart DD (1985) Grazing insects mediate algal interactions in a stream benthic ocmmunity. Oikos 44:40–46

Hill WR, Knight AW (1988) Nutrient and light limitation in two northern Californian streams. J Phycol 24:125–132

Kesler DH (1981) Periphyton grazing by *Amnicola limosa*: an enclosure-exclosure experiment. J Freshwater Ecol 1:51–59

Lamberti GA, Ashkenas LR, Gregory SV, Steinman AD (1987) Effects of three herbivores on periphyton communities in laboratory streams. J N Am Benthol Soc 6:92–104

Lamberti GA, Gregory SV, Ashkenas LR, Steinman AD, McIntire CD (1989) Productive capacity of periphyton as a determinant of plant-herbivore interactions in streams. Ecology 70:1840–1856

Lamberti GA, Resh VH (1983) Stream periphyton and insect herbivores: an experimental study of grazing by a caddisfly population. Ecology 64:1124–1135

Lentner M, Bishop T (1986) Experimental design and analysis. Valley Book Co., Blacksburg, VA

McAuliffe JR (1984) Resource depression by a stream herbivore: effects on distributions and abundances of other grazers. Oikos 42:327–333

McCormick PV (1990) Direct and indirect effects of consumers on benthic algae in an ephemeral stream food web. Can J Fish Aquat Sci 47:2057–2065

McCormick PV, Stevenson RJ (1989) Effects of snail grazing on benthic algal community structure in different nutrient environments. J N Am Benthol Soc 8:162–172

Patrick R (1970) Benthic stream communities. Am Sci 58:546–549

Pringle CM, Paaby-Hansen P, Vaux PD, Goldman CR (1986) In situ nutrient assays of periphyton growth in a lowland Costa Rican stream. Hydrobiology 134:207–213

Riber HH, Wetzel R (1987) Boundary-layer and internal diffusion effects on phosphorus fluxes in lake periphyton. Limnol Oceanogr 32:1181–1194

SAS Institute (1985) SAS user's guide: Statistics, 5th ed. Cary, NC

Sokal RR, Rohlf FJ (1981) Biometry, 2nd ed. W.H. Freeman and Co., New York

Steinman AD, McIntire CD (1987) Effects of irradiance on the community structure and biomass of algal assemblages in laboratory streams. Can J Fish Aq Sci 44:1640–1648

Steinman AD, Gregory SV, Lamberti GA, Ashkenas LR (1987) Effects of herbivore type and density on the taxonomic structure and physiognomy of algal assemblages in laboratory streams. J N Am Benthol Soc 6:175–188

Steinman AD, McIntire CD, Gregory SV, Lamberti GA (1989) Effects of irradiance and grazing on lotic algal assemblages. J Phycol 25:478–485

Stevenson RJ (1984) Procedures for mounting algae in a syrup medium. Trans An Microsc Soc 103:320–321

Stevenson RJ, Glover RM (1990) Interactive effects of algal density and current on nutrient diffusion into periphyton mats. Bull N Am Benthol Soc 7:74

Stockner JG, Shortreed KRS (1978) Enhancement of autotrophic production by nutrient addition in a coastal rainforest stream on Vancouver Island. J Fish Res Bd Canada 35:28–34

Sumner WT, McIntire CD (1982) Grazer-periphyton interactions in laboratory streams. Arch Hydrobiol 93:135–157