

Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores

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Abstract: We determined whether there were seasonal changes in the relative importance of consumers and resources in controlling stream periphyton. Our analysis included effects on algal populations and assemblage biomass and productivity. We used factorial experiments in which we manipulated snail densities, irradiance, and streamwater nutrient concentrations during two seasons, fall and spring, and compared responses with previously published summer findings. Periphyton biomass and productivity were much greater when snails were removed and nutrients and light were elevated during all seasons, indicating that all three factors were limiting or nearly limiting throughout the year. However, the relative importance of factors shifted seasonally. Irradiance limited periphyton biomass in summer and fall but not spring. In contrast, nutrients were more limiting in seasons in which light levels were higher: nutrient addition generally resulted in effects of greater magnitude in fall and spring than in summer. Snail growth was stimulated by enhanced irradiance in summer ($p = 0.06$) and by nutrient addition in fall, indicating resource limitation of both periphyton and snails. However, top-down control of periphyton by snails was also important: snails maintained low biomass assemblages dominated by only a few grazer-resistant species (e.g., basal cells of *Stigeoclonium tenue*, *Chamaesiphon investiens*) during all seasons.

Résumé : Nous avons examiné les variations saisonnières de l'importance relative des consommateurs et des ressources limitant le périphyton des cours d'eau. Notre analyse a traité des effets sur les populations d'algues et la biomasse et la productivité des communautés. Nous avons utilisé des plans d'expérience factoriels avec lesquels nous avons modifié la densité des escargots, l'éclairement et les concentrations de matières nutritives dans l'eau pendant deux saisons, l'automne et le printemps, et ensuite comparé les réactions à des résultats déjà publiés obtenus en été. La biomasse et la productivité du périphyton étaient de beaucoup supérieures en l'absence d'escargots et en présence de beaucoup de matières nutritives et de lumière cela pendant toutes les saisons, ce qui montre que ces trois facteurs étaient des facteurs limitants ou presque limitants pendant toute l'année. Par ailleurs, l'importance relative des facteurs se modifiait au cours des saisons. L'éclairement limitait la biomasse du périphyton en été et en automne mais non au printemps. Au contraire, les matières nutritives avaient un effet plus limitant au cours des saisons où l'éclairement était plus important : l'ajout de matières nutritives donnait généralement lieu à des effets de plus grande ampleur à l'automne et au printemps, comparativement à l'été. La croissance des escargots était stimulée par un plus important éclairage en été ($p = 0.06$) et par l'ajout de matières nutritives en automne, ce qui montre une limitation des ressources du périphyton et des escargots. Par ailleurs, la réduction verticale du périphyton par les escargots était aussi importante : les escargots maintenaient des communautés de faibles biomasses dominées par quelques espèces résistantes au broutage (p. ex. cellules basales de *Stigeoclonium tenue*, *Chamaesiphon investiens*) pendant toutes les saisons.

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Introduction

The balance between resource availability and consumption ultimately determines the biomass and production of trophic levels. "Bottom-up" effects of resources are predicted to be relatively more important at lower trophic lev-

els, and "top-down" effects of consumers are predicted to be relatively more important at higher trophic levels (McQueen et al. 1986; Brett and Goldman 1997; but see Osenberg and Mittelbach 1994). Both factors have been found to have effects on at least one trophic level in most cases when resource and consumer effects have been tested simulta-

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neously (Brett and Goldman 1997). Less well studied are the effects of individual population contributions to changes in biomass and productivity of trophic levels. However, some studies have shown that the relative influence of resources and consumers may depend on the specific taxa that are present as producers and consumers (Leibold and Wilbur 1992; Osenberg and Mittelbach 1994; Rosemond and Brawley 1996).

The relative influence of resources and consumers on a given trophic level can also change seasonally (Vanni and Temte 1990). In eutrophic lakes, there can be large seasonal fluctuations in phytoplankton biomass (Reynolds 1984). Vanni and Temte (1990) found that changes in phytoplankton biomass were caused by different factors during different seasons. Grazing by zooplankton reduced phytoplankton in spring, but nutrient limitation was relatively more important than grazing in the summer. There may be less seasonal variation in oligotrophic systems, where resource limitation potentially has overriding control. In oligotrophic Lawrence Lake, nutrient addition and predator removal both affected populations of fish, invertebrates, and algae, but nutrient effects were greater than those of consumers for all three trophic levels (Osenberg and Mittelbach 1994). Likewise, in the Baltic Sea, where inorganic nutrient concentrations are low, nutrient limitation was more important than grazing effects on phytoplankton biomass and productivity during the growing season (Kivi et al. 1993). In Walker Branch (WB), where this study was conducted, there is little seasonal variability in periphyton biomass (Rosemond 1994). Previous work in this stream has shown that both low availability of resources (e.g., streamwater nutrients, irradiance) and high densities of consumers (snails) maintain periphyton biomass and productivity at low levels (Steinman 1992; Rosemond et al. 1993). Similarly, periphyton was limited by light, nutrients, and grazing snails in a stream near WB (Hill et al. 1992, 1995).

In this study, we determined seasonal changes in the relative influence of nutrients, light, and snail grazing on stream periphyton in WB. Our analyses included effects on assemblage biomass and productivity and also the underlying effects on algal populations that drive the biomass and productivity response. To test hypotheses concerning temporal changes in the relative importance of different limiting factors, we manipulated levels of each of these factors (light, nutrients, snail density) in stream-side channels during three different seasons (summer, fall, spring) to reduce their potential constraints on periphyton (e.g., light and nutrients were increased, snails were excluded). The results of the summer study have been published previously (Rosemond 1993). Here, we present the results of studies conducted in the fall and spring and also include data from the summer study in a new analysis of seasonally shifting limitation of stream periphyton.

Materials and methods

Study site

The West Fork of WB is located on the Department of Energy's Oak Ridge Reservation in Oak Ridge, Tenn. (35°58'N, 84°17'W). The stream flows through a deciduous forest watershed that has been undisturbed since ca.1940. Yellow poplar (*Liriodendron tul-*

pifera L.), beech (*Fagus grandifolia* Ehrh.), and oaks (*Quercus prinus* L., *Quercus alba* L.) dominate the riparian vegetation (Johnson and Van Hook 1989). The stream is primarily spring fed, with base flow accounting for 77% of the annual stream flow (Luxmoore and Huff 1989). Average stream depth is approximately 5–10 cm and average width is approximately 1.5–3 m at base flow (Mulholland 1992).

Nutrient concentrations in WB are relatively low during all seasons ($\leq 50 \mu\text{g inorganic N}\cdot\text{L}^{-1}$, $\leq 7 \mu\text{g P}\cdot\text{L}^{-1}$) but are higher in the summer months than at other times of the year (Mulholland 1992). There is also distinct seasonality in irradiance levels, which are low in the summer months, increase following litterfall, and exhibit an annual peak in early March and April (Rosemond 1994). The most abundant herbivore in WB is the snail *Elimia clavaeformis* (family Pleuroceridae), which occurs at densities $>1000\cdot\text{m}^{-2}$ year-round (Rosemond 1994) and comprises $>95\%$ of the invertebrate biomass in the stream (Newbold et al. 1983). Weekly water temperature readings (range) during each of the experiments indicated slightly higher temperatures during summer (16°C) than during fall (9–14°C) or spring (10.5–14°C). Based on seasonal trends in potentially limiting factors, we hypothesized that light would be least limiting in the spring, when levels were highest, and nutrients would be least limiting in the summer, when their concentrations are highest. Since snails occur at consistently high densities, we hypothesized that they would constrain periphyton response to seasonal changes in resource availability.

Experimental

Two experiments were conducted in flow-through channels for approximately 7 weeks, each with approximately 2 months between experiments: (i) a fall study from 3 November 1989 to 20 December 1989 and (ii) a spring study from 7 March 1990 to 26 April 1990. The previously reported summer study was conducted from 21 July 1989 to 8 September 1989. The channels, described in Rosemond (1993), were placed next to the West Fork of WB, from which water for the channels was pumped.

The effects of light (high or ambient), nutrients (high or ambient), and snail herbivory (absent or ambient) were tested using a complete factorial design. This design resulted in eight different treatment combinations, which were duplicated and randomly assigned among 16 stream-side channels. Unglazed ceramic tiles (upper surface area = 5.3 cm²), which had been placed in WB at least 6 weeks prior to each study, were used as substrates for algal colonization in the channels. They were transferred to the channels approximately 2–3 weeks prior to the start of each experiment.

Snail densities used in the experiments were determined from bimonthly measurements in WB using a 250-cm² underwater viewer ($n = 40$ for each measurement). Grazing treatments consisted of (i) snails added at ambient densities (approximately 1100·m⁻²) and (ii) no snails. Ambient light conditions were measured monthly on cloudless days using ozalid paper light meters (Friend 1961) along a 150-m reach of stream. Light was increased in all experiments by suspending two lamps with 1000-W metal halide bulbs (Sylvania Metalarc) over the channels. Ambient light treatments were then shaded with layers of mosquito netting to simulate natural light levels. Lamps were placed on a 10-h photoperiod for both experiments.

Nutrient concentrations were increased to approximately 10 times yearly average stream concentrations by continuous addition of concentrated nutrient solutions to the high-nutrient channels (using Mariotte bottles: Peterson et al. 1983). Nutrient treatments consisted of (i) ambient (no addition) and (ii) addition of both inorganic N and P to 210 $\mu\text{g N}\cdot\text{L}^{-1}$ as NaNO₃, 40 $\mu\text{g N}\cdot\text{L}^{-1}$ as NH₄Cl, and 35 $\mu\text{g P}\cdot\text{L}^{-1}$ as K₂HPO₄ (in fall, but H₃PO₄ in spring). Water samples were taken weekly from the channels and filtered in the field through precombusted, washed glassfiber filters (Gelman type A/E, pore size = 1 μm) for nutrient analyses. Concentrations of

Table 1. Stream water nutrient concentrations ($\mu\text{g}\cdot\text{L}^{-1}$) in low- (ambient) and high-nutrient treatments in the fall (6 November to 11 December 1989) and spring (8 March to 24 April 1990) experiments.

	Ambient channels			High-nutrient channels		
	SRP	NO ₃	NH ₄	SRP	NO ₃	NH ₄
Fall						
Mean	1.8	12.0	6.3	41.2	223.0	51.9
Range	1.1–2.5	5.4–18.4	0.8–16.3	28.7–54.1	105.0–411.5	35.4–84.4
Spring						
Mean	2.4	10.1	2.0	49.8	270.2	51.0
Range	1.7–4.0	6.8–13.5	0–5.0	38.0–72.0	233.3–365.3	17.0–83.3

Note: Mean concentration is based on six weekly averages in fall and seven weekly averages in spring with ranges. Samples for weekly averages from nutrient-enriched stream-side channels ($n = 8$) and from ambient stream water ($n = 8$ for fall, $n = 1$ for spring) were taken at approximately the same time.

soluble reactive P (SRP) were measured using the ascorbic acid method (American Public Health Association 1985), ammonium N ($\text{NH}_4^+\text{-N}$) by phenate colorimetry using an autoanalyzer (Technicon TRAACS 800), and nitrate plus nitrite N ($(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$) by Cu–Cd reduction followed by automated colorimetric analysis (United States Environmental Protection Agency 1983). Carbon fixation rates were determined from an average of five tiles from each channel at the beginning and end of each experiment. Tiles were collected from the channels and placed in recirculating chambers containing 1 L of filtered (Gelman type A/E filter) water from the corresponding channel. Light levels during each incubation were maintained similar to treatment levels. $\text{NaH}^{14}\text{CO}_3$ (specific activity $0.74 \text{ MBq}\cdot\text{mmol}^{-1}$) was added to the water in each chamber and ^{14}C uptake during a 3-h incubation was determined, following extraction of cell contents in dimethyl sulfoxide and assay of ^{14}C by liquid scintillation on subsamples of the extract (Palumbo et al. 1987). Carbon fixation rates were normalized by tile area to yield area-specific productivity (ASP).

Ash-free dry mass (AFDM) of periphyton was estimated at the beginning and end of each study by collecting five tiles from each channel, placing the tiles with attached periphyton in aluminum pans, and determining differences between dry (at 60°C for 24 h) and ash (at 500°C for 24 h) masses.

Five additional tiles were collected from each channel at the beginning and end of each study to determine whether there were changes in algal community structure in response to experimental treatments. Algae were brushed from the tiles and preserved in 2% glutaraldehyde. Samples were processed with a tissue homogenizer, as required, to break up large aggregations and then sonicated. Algal units (single cells or colonies, >300 per sample) were counted and identified at $400\times$ using a Palmer-Maloney cell. Diatoms were lumped into groups or genera when counted at $400\times$. Identifications of diatoms to species were then made at $1000\times$ from permanent slides and the groups or genera initially counted were further subdivided. For each taxon, length, width, and depth or diameter of spherical cells (1–20 per taxon) were measured with an ocular micrometer and biovolume was estimated using geometric formulae modified from Kellar et al. (1980).

As an indicator of P limitation, alkaline phosphatase activity (APA) was determined on an average of five tiles from each channel at the end of each experiment. *p*-Nitrophenyl phosphate (NPP) (Sigma) was added to small jars containing filtered stream water and a tile with attached periphyton. Following a 30-min incubation, the stream water was filtered (Whatman GFF glassfiber filters) and absorbance of the filtrate was determined spectrophotometrically to determine hydrolysis of NPP (after the pH was raised to ≥ 10 by adding 0.05 mL of 1 N NaOH). In our previous analysis (Rosemond 1993), we had normalized APA per unit of chlorophyll *a*. However, because chlorophyll *a* content per cell likely changed with light treatment and season, we expressed APA here on an ar-

cal basis. APA was calculated as the amount of NPP hydrolyzed (moles of nitrophenol produced) per tile area.

Snail growth was determined as the average AFDM gain of snails from each channel during the experiment. Averages were based on $n = 85$ per channel in fall (total = 680) and $n = 20$ per channel in spring (total = 160). Individual snail widths were measured with calipers to the nearest 0.01 mm and measured snails were marked with paint and (or) bee tags (Chr. Graze, K.G., Weinstadt, Germany). Widths of each marked snail were remeasured at the end of each experiment. Increases in AFDM over the experimental periods (final average minus initial average) were determined using a width–AFDM regression computed from snails previously taken from WB ($\text{AFDM} = 1.2332 \times 10^{-5}(\text{width})^{3.98}$, $n = 50$, $R^2 = 0.96$). Growth was expressed as percent AFDM increase over the experimental period (47 days for fall, 50 days for spring).

Statistical analyses

Data were analyzed by a three-factor (nutrients, light, grazing) analysis of variance (ANOVA) using the ANOVA procedure of Statistical Analysis Systems (SAS Institute, Inc. 1985). Values used in the analyses for AFDM, ASP, and APA were means from five tiles from each replicate channel ($n = 2$ per treatment). The model that we used tested for main effects of grazing (G), light (L), and nutrients (NU) and all possible interactive effects ($G \times L$, $G \times \text{NU}$, $L \times \text{NU}$, and $G \times L \times \text{NU}$). Algal biovolumes were log transformed prior to analyses (Zar 1984). Treatment effects on biovolumes of dominant species (comprising $>10\%$ biovolume in more than nine samples) were also tested by ANOVA. Species of *Achnanthes* (*A. minutissima*, *A. lanceolata*, *A. lanceolata* var. *dubia*, *A. exigua*) were grouped for statistical tests. Snail growth data were analyzed using a two-factor (L, NU) ANOVA. For all data, if treatment effects were significant by ANOVA, post-ANOVA multiple comparison tests were conducted for each season using Ryan's *Q* test (Day and Quinn 1989). AFDM and ASP were measured before treatments were started, and ANOVAs on these data indicated no differences among treatment groups prior to the experiments.

Results

Nutrient concentrations, light levels, and snail densities

Nutrient concentrations were increased to roughly the same levels during both experiments, representing a 10- to 20-fold increase over ambient levels (Table 1). Ambient concentrations were very similar in fall and spring, and these were slightly lower than summer levels (Rosemond 1993). Irradiance levels in the stream and in ambient channels were lower in fall than in spring (Table 2). Experimental increases in irradiance were additive to ambient light, resulting in higher irradiance in “high-light” channels in spring than in

Table 2. Light levels (photosynthetically active radiation, $\text{mmol quanta}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$) in the stream and in stream-side channels.

Date	Stream	Ambient channels	High-light channels
Fall			
9 Oct. 1989	517 (62) (<i>n</i> = 12)		
18 Nov. 1989		490 (<i>n</i> = 1)	7334 (<i>n</i> = 1)
30 Nov. 1989	1251 (70) (<i>n</i> = 10)	1216 (<i>n</i> = 1)	8356 (2358) (<i>n</i> = 2)
26 Jan. 1990	1199 (51) (<i>n</i> = 12)		
Spring			
4 Mar. 1990	4234 (90) (<i>n</i> = 12)		
4 Apr. 1990	4267 (92) (<i>n</i> = 11)	2700 ^a	11 830 (558) (<i>n</i> = 3)
24 Apr. 1990	2121 (96) (<i>n</i> = 12)		
30 May 1990	526 (81) (<i>n</i> = 11)		

Note: Data are means (SE) (number of samples).

^aEstimated from instantaneous readings.

Table 3. Treatment effects on periphyton biomass as AFDM and ASP.

Treatment	df	AFDM	ASP
Fall			
G	1	(-) 94.80****	(-) 72.24****
L	1	(+) 7.23*	(+) 11.22**
NU	1	(+) 48.85****	(+) 77.14****
G × L	1	21.16**	21.01**
G × NU	1	32.88***	47.56****
L × NU	1	6.60*	24.70***
G × L × NU	1	12.42***	31.27***
Spring			
G	1	(-) 13.77**	(-) 150.62****
L	1	ns	(+) 23.40**
NU	1	(+) 9.96**	(+) 152.59****
G × L	1	ns	15.16**
G × NU	1	8.13*	72.78****
L × NU	1	ns	20.99**
G × L × NU	1	ns	11.22**

Note: Data are *F* values from ANOVA of week 7 measurements. **p* ≤ 0.05, ***p* ≤ 0.01, ****p* ≤ 0.001, *****p* ≤ 0.0001; ns, not significant. Treatment effects: G, grazing; L, light; NU, nutrients. + and - indicate the direction of the treatment effect.

fall. Ambient light levels were maintained at a constant level during the spring experiment, when light levels in the stream were fairly constant. During the fall experiment, two light levels were used, low levels initially (similar to summertime levels) and then higher levels approximately 3 weeks after the start of the experiment (starting on 28 November 1989), to simulate the ambient increases in light following leaf fall.

Snail densities in the stream were fairly constant over the study period (Rosemond 1994), and experimental densities used in grazed channels were similar to those in the stream ($1042 \text{ snails}\cdot\text{m}^{-2}$ in fall and $1103 \text{ snails}\cdot\text{m}^{-2}$ in spring). Den-

sities of snails observed foraging on tile surfaces were somewhat lower than stocking densities (snails not on tiles were on channel walls or in crevices between tiles). The mean of counts on tile surfaces made during the fall was $354 \text{ snails}\cdot\text{m}^{-2}$ (range 256–561, *n* = 7), approximately one-third of the densities initially stocked. These counts were not made during the spring study, but conditions were similar to those in the fall. Any observed snail immigrants were removed from ungrazed channels every 1–2 days. Low densities of mayflies (primarily *Baetis* spp.) colonized channels but were also removed every 1–2 days when present.

Effects on periphyton biomass and productivity

Snail grazing had negative effects and nutrient addition had positive effects on periphyton AFDM in both the fall and spring (Table 3; Fig. 1). In addition, significant G × NU interactions were likely the result of greater effects of nutrients when grazers were absent. Enhanced light had positive effects on AFDM in the fall but not in the spring (Table 3). All treatment effects and interaction terms on ASP in both seasons were significant (Table 3), with the highest ASP observed in ungrazed/+NU&L treatments (Fig. 2).

Effects on index of P limitation

Nutrient additions had a negative effect on APA during spring and fall (Fig. 3; Table 4). Although APA was also generally lower with nutrient addition in summer, high variability resulted in no significant effect of nutrient addition. APA was also reduced by grazing in fall but not during the other two seasons (Table 4).

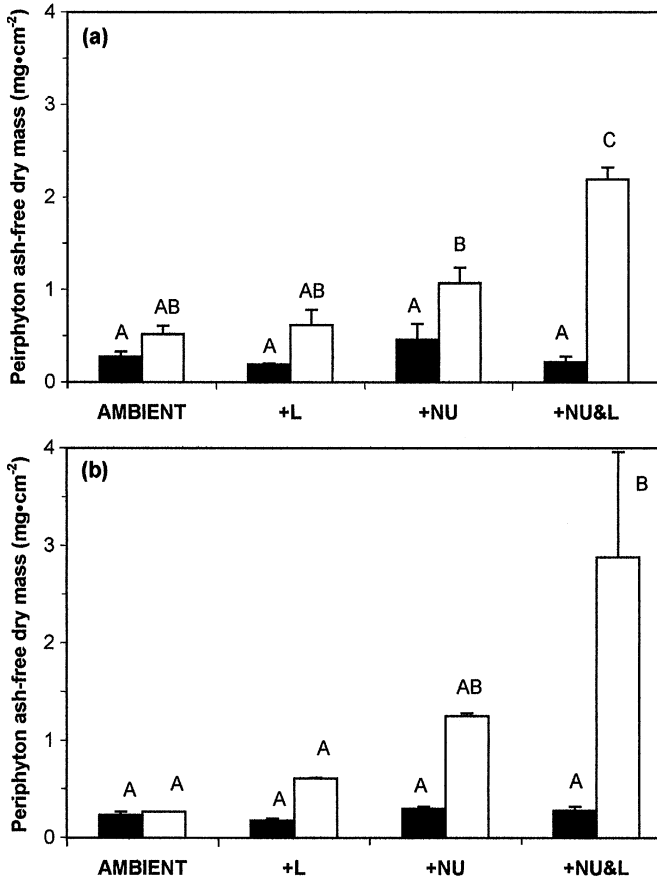
Effects on algal populations

In general, those species that persisted in the presence of grazers were not those that responded to increases in resources when grazers were removed. Grazed communities were composed largely of basal cells of *Stigeoclonium tenue* (Kuetz.) (Chlorophyta) and *Chamaesiphon investiens* (Cyanophyta) in spring and fall (Figs. 4a and 5a). Grazing had positive effects on *Chamaesiphon* biovolume, and increased irradiance had no effect (during spring) or negative effects (during fall) on both *Chamaesiphon* and *Stigeoclonium* biovolumes (Table 5). In contrast, species that dominated communities when grazers were removed increased in biovolume in response to increased resources. Ungrazed communities were composed of several diatom species (predominately *Melosira varians* (Ag.), *Meridion circulare* (Grev.), *Cocconeis placentula* (Ehr.), *Achnanthes* (Bory) spp., and *Eunotia pectinalis*) in both spring and fall (Figs. 4b and 5b). Grazing had negative effects and increased resources (light and nutrients) had positive effects on the dominant diatom taxa (Table 5). These species responded positively to increased irradiance in fall but less so in spring (Table 5).

Effects of nutrients and light on snail growth

Nutrient addition had significant positive effects on snail growth in the fall (results of two-way ANOVA: light effect not significant, nutrient effect significant at *p* < 0.05). Snail growth rates were generally higher in +NU&L treatments in both seasons (Fig. 6). No statistically significant effects of nutrient addition or enhanced light were observed in the

Fig. 1. Periphyton AFDM in different treatments during the last week of the (a) fall experiment (3 November to 20 December 1989) and (b) spring experiment (7 March to 26 April 1990). Ambient: shaded, low-nutrient treatments; +L, high-light, low-nutrient treatments; +NU, shaded, high-nutrient treatments; +NU&L, high-light, high-nutrient treatments. Solid bars, grazed (snails added at ambient densities); open bars, ungrazed (no snails added). Lines above bars represent ± 1 SE. Means of bars with the same letter are not significantly different by post-ANOVA multiple comparison tests. Note that the AFDM values in Rosemond (1993) are an order of magnitude lower than they should be due to a calculation error.



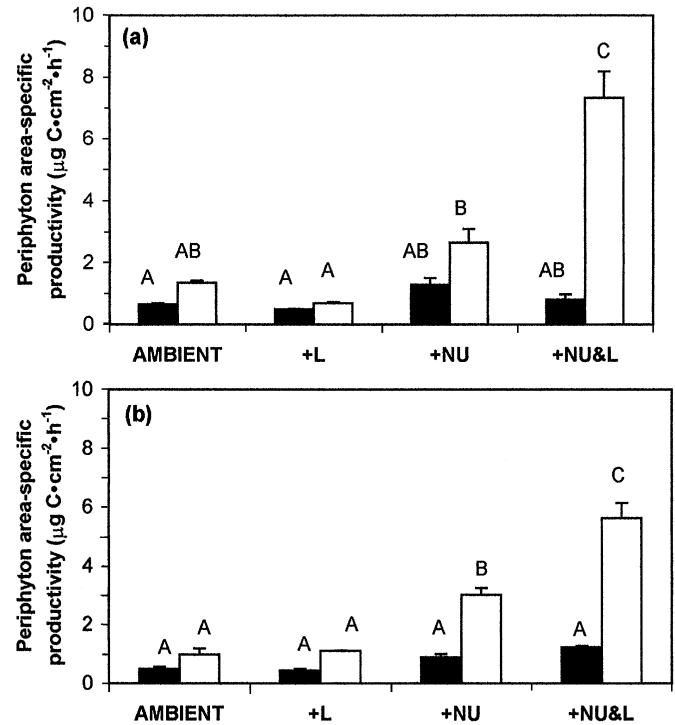
spring. Comparison of the magnitude of daily growth rates ($\mu\text{g}/\text{day}$ (SD)) by season indicates the highest growth rates and greatest overall response to resource addition in summer. Daily growth rates in ambient, +L, +NU, and +NU&L treatments in fall were 2.87 (0.15), 1.91 (0.90), 3.72 (0.75), and 4.79 (0.75); in spring, 1.90 (2.12), 7.70 (0.71), 7.20 (3.68), and 10.40 (4.52); and in summer, 7.04 (3.61), 13.67 (1.15), 10.92 (6.78), and 17.55 (1.15). In all seasons, the highest snail growth rates were observed in treatments in which both irradiance and nutrient concentrations were elevated.

Discussion

Differences in the effects of grazing, light, and nutrients among seasons

During both fall and spring, periphyton biomass and pro-

Fig. 2. Area-specific periphyton productivity in the different treatments during the last week of the (a) fall experiment and (b) spring experiment. Treatments and symbols as in Fig. 1.

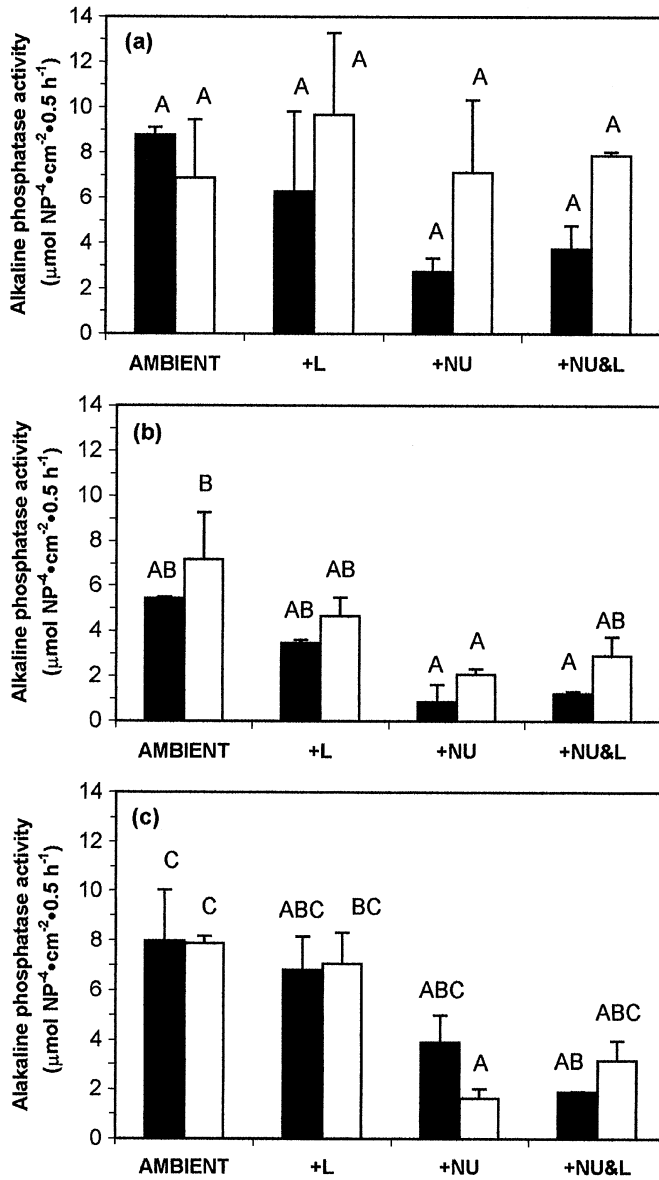


ductivity were considerably greater when snails were removed and light and nutrients were elevated together than when any one or two of these factors were manipulated. Similar results were obtained in the summer study reported earlier (Rosemond 1993). This suggests that all three of these factors were at or near limiting levels year-round. These results contrast with those of Vanni and Temte (1990), indicating a clear shift from limitation due to consumers in spring to limitation due to resources in summer. In WB, both consumers and resources appear to be important constraints on periphyton, regardless of season.

Although all three factors were at or near limiting levels during different seasons, their relative importance as constraints on periphyton changed somewhat with season. This appears to be a result of changes in the prevailing nutrient and light conditions. There were positive effects of light on periphyton biomass and biomass of several algal populations in the fall but not in the spring when irradiance was already sufficiently high. The effects of enhanced light, however, appeared to be greatest in summer, when increased irradiance resulted in the greatest increase in periphyton biomass (in ungrazed/+L treatments, compared with other seasons) and enhanced light increased snail growth (Rosemond 1993).

The response of periphyton biomass and productivity to nutrient addition was greater in seasons when light levels were greater. There were significant positive effects of nutrient addition during all seasons, but the magnitude of the effect of nutrients on biomass and productivity (in ungrazed treatments) was greater in fall and spring than in summer (compare with Rosemond 1993). Significant positive effects of nutrient addition on snail growth were only observed in

Fig. 3. APA in different treatments during the last week of the (a) summer experiment, (b) fall experiment, and (c) spring experiment. Treatments and symbols as in Fig. 1.



the fall; however, the magnitude of the effect of nutrient addition (compared to ambient conditions) on daily growth rates was actually greater in spring than in fall (mean difference of 5.3 $\mu\text{g}/\text{day}$ in spring and 0.85 $\mu\text{g}/\text{day}$ in fall between treatments). Other studies have shown that the effects of nutrient addition on stream periphyton are dependent on prevailing light conditions and that effects of irradiance are dependent on ambient nutrient concentrations (see references cited in Rosemond 1993). The results of our study further demonstrate that effects of nutrients and light on algal communities can be highly interdependent. Seasonal increases in light or nutrients may only have positive effects on algal communities that are not constrained by the other factor.

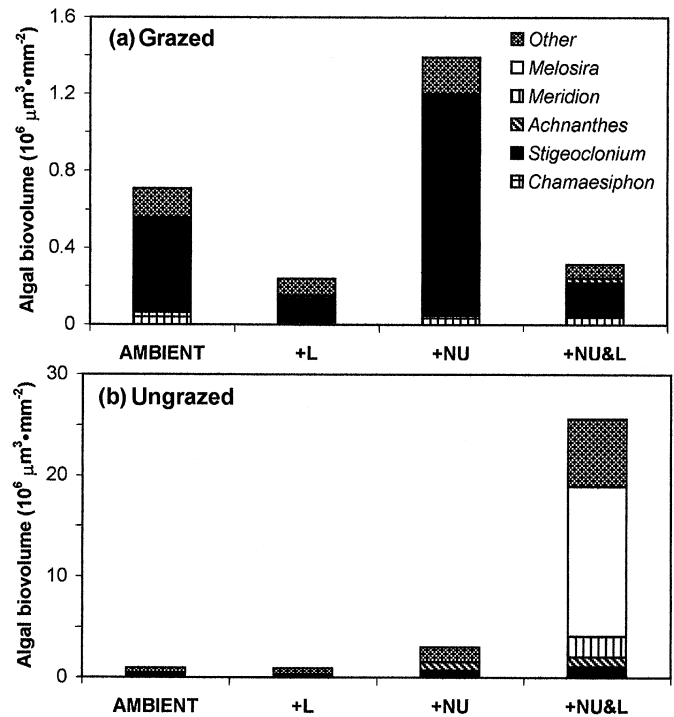
In contrast with the effects of light and nutrients, which changed with season, snails appeared to have very similar

Table 4. Results of ANOVA on APA from summer, fall, and spring experiments.

Treatment	df	Summer	Fall	Spring
G	1	ns	(-) 5.70*	ns
L	1	ns	ns	ns
NU	1	ns	(-) 28.67***	(-) 38.08***
G \times L	1	ns	ns	ns
G \times NU	1	ns	ns	ns
L \times NU	1	ns	4.70†	ns
G \times L \times NU	1	ns	ns	ns

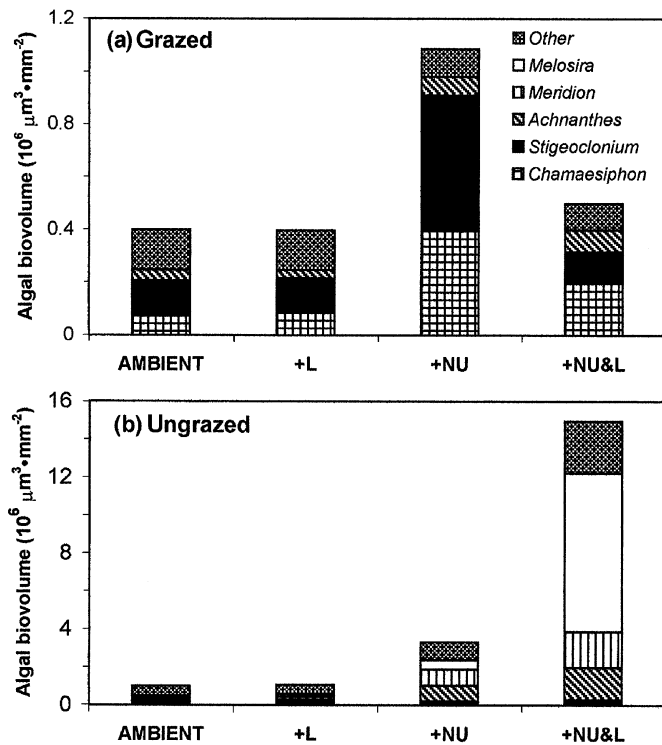
Note: Here, we present results of APA normalized per area, in contrast with the results from the summer study previously reported in Rosemond (1993), where values were normalized by chlorophyll *a*. † $p \leq 0.10$, * $p \leq 0.05$, *** $p \leq 0.001$; ns, not significant. Treatment effects: G, grazing; L, light; NU, nutrients. - indicates the direction of the treatment effect.

Fig. 4. Algal species composition of (a) grazed and (b) ungrazed treatments at the end of the fall experiment based on biovolumes of *Chamaesiphon investiens*, *Stigeoclonium tenue*, *Achnanthes* spp., *Meridion circulare*, *Melosira varians*, and all other algal species. Note the different axes for the grazed and ungrazed treatments. Treatments and symbols as in Fig. 1. Note that *M. circulare* was misidentified as *Peronia intermedium* in two previous publications: Rosemond (1993) and Rosemond et al. (1993).



effects on periphyton, regardless of season. In this study, increases in nutrients and light were ultimately manifested in increased snail biomass, indicating that snails were able to harvest increases in algal productivity and maintain low algal biomass even when resources were increased. These experimental results corroborate field observations that biomass and productivity of periphyton changed very little seasonally, despite large seasonal changes in light levels and nutrient concentrations (Rosemond 1994).

Fig. 5. Algal species composition of (a) grazed and (b) ungrazed treatments at the end of the spring experiment based on biovolume. Species designations, treatments, and symbols as in Fig. 4. Note the different axes for the grazed and ungrazed treatments.



Different algal species were abundant under grazed conditions regardless of nutrient and light levels (e.g., *Chamaesiphon* and *Stigeoclonium*) than when resources were increased and herbivores were removed (e.g., *Melosira* and *Meridion*). These results partially contrast with predictions of Osenberg and Mittelbach (1994) that the species composition of plants and prey should change across productivity gradients. In our study, algal species composition was largely constant over gradients of light and nutrients: algal populations were controlled more by the presence or absence of herbivores than by changes in resources. The changes in algal species composition that we observed are consistent with community structure being determined by "trade-offs" between herbivore resistance and competitive abilities, as has been suggested for other plant communities (Olsen and Lubchenco 1989; Huntly 1991). Manifestations of this trade-off (e.g., grazer-resistant plant species being overgrown by faster-growing species when herbivores were removed) have been observed previously in streams (Power et al. 1988; Rosemond et al. 1993), coral reefs (Hay 1981; Lewis 1986), intertidal communities (Lubchenco 1978; Steneck 1982), and terrestrial plant communities (Huntly 1987). Thus, algal populations that had high growth potential, such as the dominant diatom taxa observed in ungrazed treatments in this study, could respond to increases in resources, but only when grazers were excluded. WB may be a system that is chronically at the upper end of a spectrum of grazing intensity. In this study, and other studies in WB, herbivores were most important in determining community structure and largely constrained periphyton response to

increased resources. However, in other systems where grazing pressure is lower, and as evidenced to some extent in ungrazed treatments in this study, species composition may change in response to gradients in resources (e.g., Osenberg and Mittelbach 1994).

Changing effects of stream herbivores under different resource conditions

Whereas snails had pronounced effects on species composition under ambient resource conditions, there was very little increase in biomass or productivity of periphyton when snails were removed without additional amendments of light or nutrients. In contrast, differences in biomass and productivity between grazed and ungrazed treatments in which light and nutrients were increased were great. Similarly, when both herbivores and growth-limiting resources have been manipulated in other streams, grazer effects were greatest at high resource levels (Feminella and Hawkins 1995).

These results differ from those of other studies that have found reduced effects of grazers under high resource conditions (e.g., Hansson 1992). In Hansson's (1992) study, grazers became less effective as lake water nutrient concentrations increased. In contrast, our findings appear to be a result of (i) herbivores being able to maintain low plant biomass under high resource conditions coupled with (ii) an increase in plant productivity in response to increased resource levels when herbivores were removed. Thus, the conditions that determine whether herbivores have reduced or increased effects on plant communities when resources are elevated may depend on whether or not herbivores are food limited and (or) plants are resource limited. Food limitation of consumers (e.g., Hill et al. 1995) and resource limitation of plants (e.g., Pringle 1987) may be common in streams. These conditions may result in relatively minor effects of herbivores on the biomass and productivity of plant communities under ambient conditions but greater effects when growth-limiting resources become abundant (e.g., storm-influenced nutrient pulses, seasonal changes in light availability).

What limits primary producers in streams? An analysis of proximate constraint

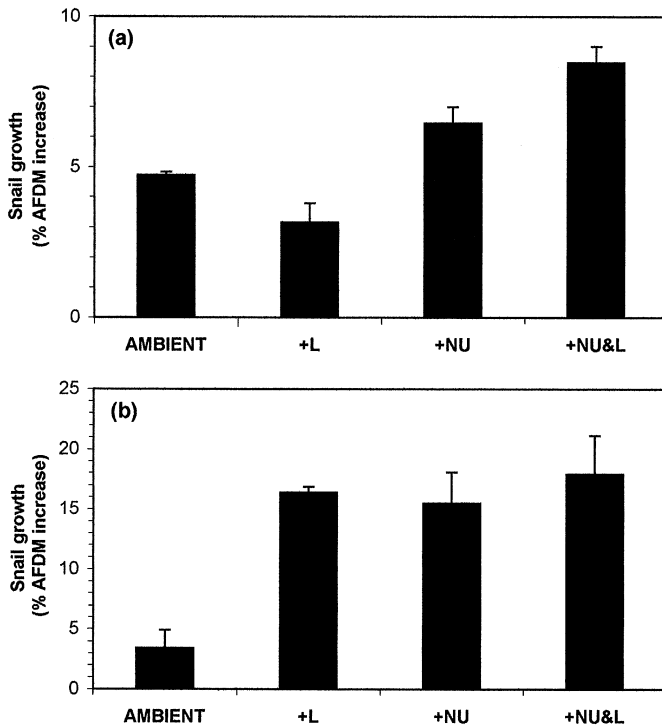
These studies illustrate that limitation is a relative term. Here, and in Rosemond (1993), any of the factors tested could only be considered to be primarily limiting when growth constraints due to other factors were removed. For example, for AFDM, nutrient addition had no effect with grazers present under ambient light conditions in the summer, but had a large effect when grazers were removed and light levels were elevated. Likewise, removal of grazers in the spring had no appreciable effect on periphyton biomass, but had substantial effects when nutrients were simultaneously increased. Interestingly, not one of these factors would have been identified as "limiting," had their effects been tested singly, as indicated by multiple comparison tests for differences in means for single-factor manipulations (e.g., grazed/ambient versus ungrazed/ambient or grazed/ambient versus grazed/+NU), despite the fact that 4- to 10-fold increases over ambient levels for most parameters were observed when all three factors were manipulated.

Table 5. Treatment effects on the biovolume of different algal species.

Treatment	df	CHAM	STIG	ACH	MERI	MELO
Fall						
G	1	(+) 11.04**	ns	(-) 314.17****	(-) 27.22***	(-) 16.72**
L	1	(-) 4.81†	(-) 10.99**	(+) 12.01**	(+) 4.98†	(+) 4.37†
NU	1	ns	(+) 14.93**	(+) 36.08***	ns	(+) 4.37†
G × L	1	ns	4.74†	11.91**	ns	4.37†
G × NU	1	ns	ns	49.74****	ns	4.37†
L × NU	1	ns	ns	8.35*	ns	16.72**
G × L × NU	1	4.25†	ns	3.59†	ns	16.72**
Spring						
G	1	(+) 7.06*	ns	(-) 58.74****	(-) 6454.49****	(-) 10.21**
L	1	ns	ns	ns	(+) 5.82*	ns
NU	1	ns	ns	(+) 31.11***	(+) 64.99****	(+) 3.89†
G × L	1	ns	ns	ns	5.82*	ns
G × NU	1	ns	ns	7.84*	64.99****	3.89†
L × NU	1	ns	ns	ns	ns	ns
G × L × NU	1	ns	ns	ns	ns	ns

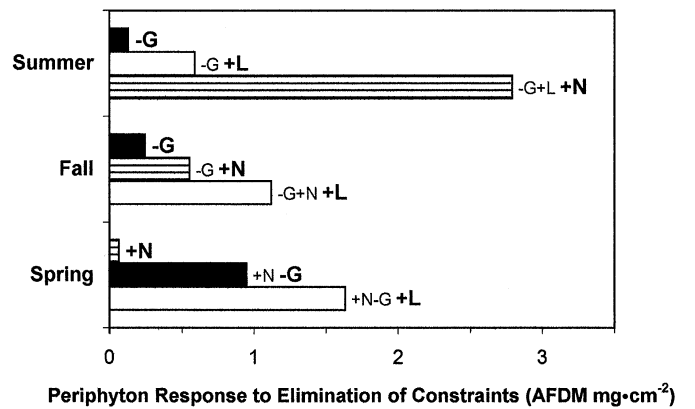
Note: Data are *F* values from ANOVA of log biovolume of *Chamaesiphon investiens* (CHAM) (division Cyanophyta), *Stigeoclonium tenue* (STIG) (division Chlorophyta), *Achnanthes* spp. (ACH), *Meridion circulare* (MERI), and *Melosira varians* (MELO). *Achnanthes* spp., *Meridion*, and *Melosira* are all diatoms (division Chrysophyta, class Bacillariophyceae). †*p* ≤ 0.10, **p* ≤ 0.05, ***p* ≤ 0.01, *****p* ≤ 0.001, ******p* ≤ 0.0001; ns, not significant. Treatment effects: S, season; G, grazing; L, light; NU, nutrients. + and - indicate the direction of the treatment effect.

Fig. 6. Snail growth as percent increase over the (a) fall and (b) spring experimental periods. Treatments as in Fig. 1.



Periphyton biomass in WB appears to be constrained by colimitation of several factors. Nonetheless, each of these factors is not of equal importance. By examining the relative response to single- and combined-factor manipulations, we determined the order of importance of these factors as constraints on periphyton. In this way, we assessed whether the proximate limitation of periphyton biomass in this stream during different seasons was the high abundance of snails or

Fig. 7. Analysis of order (top to bottom within each season) of proximate limitation by nutrients (pinstriped bars), light (open bars), and grazing (solid bars) on periphyton AFDM. The length of each bar represents the increase in AFDM due to the removal of constraints due to each particular factor. Factors listed second or third for each season include effects of factors listed above them. See text for further explanation.



the low levels of light or nutrients. To do this, we determined which single constraint, when removed, resulted in the greatest increase in biomass or productivity under ambient levels of the other two factors. This analysis was conducted for periphyton AFDM and the results are depicted in Fig. 7. Each factor was assigned a position (top to bottom for each season) based on its proximate control of AFDM. The top factor is that which was closest to being the single most important factor in controlling AFDM during that season (it yielded the greatest increase in AFDM when its level was manipulated alone). The second factor in each vertical set yielded the greatest increase after the first factor had been increased (or removed, in the case of snails). For example, in the fall, removal of snails resulted in greater increases

in AFDM over ambient, grazed levels ($+0.24 \text{ mg}\cdot\text{cm}^{-2}$) than an increase in nutrients ($+0.18 \text{ mg}\cdot\text{cm}^{-2}$) or an increase in light ($-0.08 \text{ mg}\cdot\text{cm}^{-2}$). Then, considering ungrazed treatments, increases in nutrients ($+0.56 \text{ mg}\cdot\text{cm}^{-2}$) resulted in greater increases in AFDM than increases in light ($+0.10 \text{ mg}\cdot\text{cm}^{-2}$). Finally, increasing light resulted in an AFDM increase of $1.12 \text{ mg}\cdot\text{cm}^{-2}$ under elevated nutrient conditions in the absence of grazing. Note that although increases in light after snails were removed and nutrients were increased resulted in the greatest periphyton AFDM response, light was the least important proximate limiting factor during the fall. The patterns of proximate limitation differed among seasons; factors were important in the order of G, L, and NU in the summer, G, NU, and L in the fall, and NU, G, and L in the spring. The same analysis was conducted for total algal biovolume for which similar results were obtained (factors were important in the order of G, L, and NU in the summer, NU, G, and L in the fall, and NU, G, and L in the spring).

The results of the preceding analyses help to illustrate the manner in which multiple constraints control periphyton in WB during different seasons. The initial hurdle to an increase in biomass in the summer and fall is to overcome losses due to snail grazing. Light was secondarily limiting during the summer, whereas nutrients were secondarily limiting during the fall. Nutrients were initially the most important constraint in the spring, but once supplied at sufficient concentrations, snail grazing limited biomass accrual. Nutrients were the least important constraint in the summer (when concentrations in stream water were highest), and light was the least important constraint during the fall and spring (when ambient light levels were highest), consistent with our a priori hypotheses.

A somewhat surprising result was that removal of grazers generally had a greater proximate effect on algal biomass than resource addition, although the ultimate effects of grazers in constraining periphyton response to resources had been predicted. Why were proximate effects of grazers so great? They were probably due to the identity of both the producers and consumer. Previous studies have shown that at least one of the grazer-resistant taxa in this study (*S. tenue*) has a lower biomass-specific productivity rate than an assemblage of diatom taxa, similar to the assemblages that were observed in ungrazed treatments in these studies (Rosemond and Brawley 1996). When grazers were removed, taxa with higher inherent rates of productivity replaced slow producers. In addition, the identity of the herbivore in this system is also clearly important. Many previous studies have shown negative effects of snails, including *E. clavaeformis*, on the biomass of stream algae (Feminella and Hawkins 1995).

Results of this study indicate that interactions between resources and consumers constrained biomass and productivity of stream periphyton. But are resources and consumers both important in controlling periphyton in other streams as well? Hydrologic disturbance, which was not considered in this study, can be a particularly powerful structuring force in streams. Many studies have found that hydrologic factors correlate with seasonal patterns of lotic primary producers (Wehr 1981; Duncan and Blinn 1989; Júnior et al. 1991). Other studies have found variation in resources to be most

important in determining seasonal patterns in stream periphyton (Sumner and Fisher 1979; Keithan and Lowe 1985; Sheath and Burkholder 1985). Hydrologic disturbances, common in many streams, can also interact with resources or consumers to determine seasonal patterns. For example, Power (1992) has shown that the algae in some northern California rivers shift from being under hydrologic control during the winter wet season to consumer control during the dry season. Grimm and Fisher (1986) suggested that resource (nutrient) availability is important in algal regrowth following depression of biomass due to flood disturbance. Our study was conducted in a spring-fed stream, where disturbance due to flooding may be less important than in more hydrologically variable streams. Our results indicate that both consumption and resource limitation were important constraints on periphyton year-round and agree with predictions that biotic interactions can be important structuring factors in relatively benign environments such as spring-fed streams (Peckarsky 1983).

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