Phosphorus and light colimit periphyton growth at subsaturating irradiances

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SUMMARY

1. This study investigated the combined effects of light and phosphorus on the growth and phosphorus content of periphyton. To investigate the potential for colimitation of algal growth by these two resources, diatom-dominated periphyton communities in large flow-through laboratory streams were exposed under controlled conditions to simultaneous gradients of light and phosphorus.

2. Periphyton growth rate was predictably light-limited by the subsaturating irradiances (12–88 μmol photons m^{-2} s^{-1}) used in this experiment. However, phosphorus concentration also limited growth rate: growth increased hyperbolically with increasing soluble reactive phosphorus (SRP), reaching a threshold of growth saturation between 22 and 82 μg L^{-1}.

3. Periphyton phosphorus content was strongly and nonlinearly related with SRP, reaching a maximum at 82 μg L^{-1} SRP. Contrary to the Light : Nutrient Hypothesis, periphyton phosphorus content did not decrease with increasing light, even at the lowest concentrations of SRP. Periphyton phosphorus was highly correlated with periphyton growth rate (Spearman's $\rho = 0.63$, $P < 0.005$).

4. Multiple regression analysis reinforced evidence of simultaneous light and phosphorus limitation. Both light and periphyton phosphorus content were significant variables in multiple regressions with growth parameters as dependent variables. Light alone accounted for 67% of the variance in periphyton biomass, and the addition of periphyton phosphorus as an additional independent variable increased the total amount of variance explained to 81%.

5. Our results did not support the hypothesis that extra phosphorus is required for photoacclimation to low light levels. Rather, the effect of additional phosphorus may have been to accommodate increased requirements for P-rich ribosomal RNA when growth was stimulated by increased light. The potential colimitation of periphyton growth by phosphorus and light at subsaturating irradiances has important implications in both theoretical and applied aquatic ecology.

Keywords: benthic algae, colimitation, light, phosphorus, stoichiometry

Introduction

Light and nutrients are two of the most important variables affecting algal growth in aquatic ecosystems.

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Much research has been devoted to quantifying the individual effects of these variables, resulting in the establishment of well-known nonlinear relationships between algal photosynthesis and light (e.g. Jassby & Platt, 1976) and between algal growth and nutrients (e.g. Caperon, 1967). Much less is known about the combined effects of these two variables, especially when they are both potentially limiting. Light and

nutrients vary considerably across and within natural ecosystems, and the simultaneous scarcity of both resources is common. In lotic ecosystems, attached algae are frequently exposed to both low photon flux density (caused by the shade of riparian vegetation) and low concentrations of phosphorus or nitrogen in oligotrophic streams (e.g. Hill & Knight, 1988; Hill & Harvey, 1990; Rosemond, 1993). Although the potential for colimitation of algal growth by both light and nutrients exists in many oligotrophic habitats, few studies have addressed colimitation (but see Fahnenstiel, Scavia & Schelske, 1984; Healy, 1985; Fahnenstiel *et al.*, 2000). The prospect that nutrients may affect the growth of algae that are already light-limited has both conceptual and applied relevance.

Threshold responses are frequently used to describe the effects of limiting resources. When the limiting resources are nutrients, Liebig's Law of the Minimum predicts that only one nutrient limits growth at any particular time (e.g. Droop, 1974). Accordingly, a second nutrient in short supply should become growth-limiting only after the supply of the first nutrient approaches a saturation threshold. This idea of sequential limitation has also been applied when non-nutrient resources are in short supply (Davis, 1976). The failure of nutrient enrichment to stimulate algal growth in some highly shaded streams has been attributed to subsaturating irradiances (e.g. Lowe, Golladay & Webster, 1986; Hill & Knight, 1988; Mosisch, Bunn & Davies, 2001). Nonetheless, it remains unclear if algal growth is exclusively limited by a single factor when both a physical factor (e.g. light and temperature) and a nutrient (e.g. phosphorus) are in short supply (Healy, 1985). Simultaneous limitation by two or more resources can theoretically occur, if the availability of one resource facilitates the acquisition of another. For example, algae are capable of increasing their light capture efficiency in low light by increasing cellular chlorophyll-*a* concentration, which in turn requires a higher concentration of nitrogen (Rhee & Gotham, 1981; Geider, MacIntyre & Kana, 1998). It has been suggested that phosphorus may also be required in higher amounts for algae to photoadapt to low light (e.g. Hessen, Færøvig & Andersen, 2002; Dickman, Vanni & Horgan, 2006).

The Light : Nutrient Hypothesis predicts that algal nutrient content will increase with increasing nutrients in the water column but decrease with increasing light intensity (Sterner *et al.*, 1997). The negative effect of light on algal nutrient content is hypothesized to occur because: (i) carbon in excess of that needed for growth accumulates in cells of algae that are photosynthesizing vigorously under high light conditions and (ii) additional nutrients are required by algal cells photoadapting to low light conditions (e.g. Hessen *et al.*, 2002; Dickman *et al.*, 2006). Simultaneous experimental manipulations of light and nutrients in the laboratory or in open-water has generally supported predictions of the Light : Nutrient Hypothesis, showing that phytoplankton phosphorus content and the growth of herbivorous zooplankton are positively related with dissolved phosphorus and negatively related with light (e.g. Urabe & Sterner, 1996; Hessen *et al.*, 2002). Empirical support for the Light : Nutrient Hypothesis in benthic habitats has been mixed, however (Frost & Elser, 2002; Hillebrand, de Montpellier & Liess, 2004).

We examined the combined effects of light and phosphorus on the growth and phosphorus content of lotic periphyton. Periphyton includes bacteria, fungi and microfauna in addition to algae, but algae typically dominate both in biomass and metabolism (e.g. Neely & Wetzel, 1995; Carr, Morin & Chambers, 2005). Here, we exposed periphyton to simultaneous gradients of light and phosphorus to: (i) explore the potential of colimitation of algal growth by light and phosphorus; (ii) identify thresholds of phosphorus-limited algal growth and (iii) test the Light : Nutrient Hypothesis of algal stoichiometry. The results of these simultaneous manipulations of both light and nutrients have important implications for establishing nutrient standards for streams and for predicting the impacts of limiting resources on the food quality of consumers.

Methods

Experimental streams

This study was performed in five indoor laboratory streams at the Oak Ridge National Laboratory (ORNL), Tennessee. The *U*-shaped, flow-through streams are 22-m long and 0.3-m wide, and are supplied with water from First Creek, an unpolluted first order stream on the Oak Ridge Reservation. Substrata in the streams consisted of continuous mats

of unglazed, white ceramic tiles (each tile was $2.4 \times 2.4 \times 0.6$ cm^3), and illumination was provided by eight metal halide lamps (400 W) positioned above each stream. A 14:10 hours light : dark cycle was maintained throughout the experiment with a timer wired to the lamp circuits. Water flow in the streams was initiated 3 weeks before experimental manipulations to allow periphyton communities to develop. Tiles in the 11-m downstream sections of the streams were scrubbed the day before the experimental treatments were applied, but periphyton was left undisturbed on the tiles in the upstream ends to provide a source of algal colonists.

Experimental treatments

Five phosphorus concentrations were randomly applied to the five streams by dripping a stock solution of dissolved phosphorus at the head of each stream with a Mariotte bottle. A different stock solution of dissolved Na$_2$HPO$_4$ was used for each stream; these solutions were added at rates calculated to achieve target concentrations of 6, 12, 25, 75 or 150 µg L^{-1} phosphorus in the streams. The stream with the lowest targeted concentration (6 µg L^{-1}) received a drip without phosphorus, so the concentration in that stream was the ambient phosphorus concentration in First Creek (estimated to be 6 µg L^{-1}). Nitrate (NaNO$_3$) was also added to the stock solutions to ensure that nitrogen did not limit periphyton growth. The target nitrate concentration was 300 µg L^{-1}, a concentration of inorganic nitrogen that Rier & Stevenson (2006) found to saturate periphyton accrual in streams. Drip rates were checked daily and adjusted as needed, as were discharge rates in the streams. Average discharge per stream was 0.23 ± 0.02 (SD) L s^{-1}. The amount of stock solution used during the experiment was recorded for each stream.

Four light treatments were applied to the downstream half (11 m) of each stream. Randomly chosen 1-m sections of each stream were shaded with 0, 1, 2 or 3 layers of black plastic window screening (Fig. 1). Each layer of screening reduced irradiance on the floor of the stream by approximately 50%. Shaded sections were separated by approximately 1-m unscreened sections. Light and phosphorus treatments began on 12 May and the experiment ended on 22 May 2005.
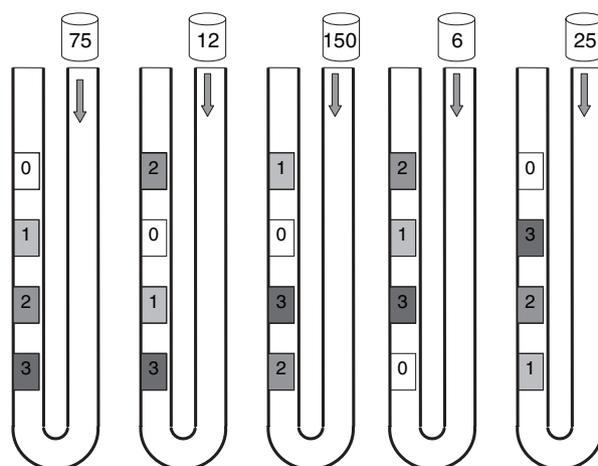

Fig. 1 Schematic of experimental stream setup. Numbers in cylinders at the head of each stream refer to target phosphorus concentration in the stream. Numbers in boxes in the downstream portion of each stream refer to the number of screen layers.

Sampling and analysis

Periphyton-covered tiles were removed daily for measurements of dry mass and periphyton phosphorus from random locations beneath each light treatment beginning on the third day of the experiment. The tiles were immediately placed on ice in reduced light until the last sample was taken. Irradiance was measured with a quantum sensor at the specific location of each tile that was removed for analysis. Periphyton was brushed from the tiles and filtered onto pre-weighed Whatman GFF filters (Whatman Inc., Florham Park, NJ, U.S.A.). The filters were dried overnight at 60 °C and weighed to the nearest 0.01 mg. The net biomass-specific growth rate (µ) for each light × phosphorus treatment combination was determined by fitting the exponential growth equation $y_t = ae^{\mu t}$ to the time series of periphyton dry mass. In this equation, y = dry mass at time t, a, the initial dry mass, µ, the biomass specific growth rate (day^{-1}) and t, the time (days). Because the calculation of µ does not include losses from sloughing/emigrating cells or grazing by microherbivores, it is a conservative estimate of biomass-specific growth rate. Mean r^2 for the 20 growth rate regressions was 0.87.

Phosphorus content was analysed in the periphyton collected on the last day of sampling. Filters were cut in half after their dry mass was determined (described above), weighed, ashed at 480 °C and weighed again. The filters were then placed in vials containing 5 mL

of 1 N HCl. These vials were heated at 80 °C for an hour to extract phosphorus from the filters. The acid extracts were diluted to 50 mL with double-deionized water, and the phosphorus in the diluted solution was analysed by the ascorbic acid method (APHA, 2005). Filter blanks and standard reference material (tomato leaves, NIST SRM 1573a) were analysed concurrently with the periphyton; recovery of phosphorus from the SRM was 98%.

Stream water was sampled 2, 7 and 10 days after the start of the experiment. Samples were taken with a syringe just upstream of the downstream experimental section, filtered through a Whatman GFF filter and frozen until analysis. Soluble reactive phosphorus (SRP) was analysed by the ascorbic acid method (APHA, 2005) with a 5-cm cell, and nitrate was analysed by second-derivative spectroscopy (Crumpton, Isenhart & Mitchell, 1992). Temperature was measured throughout the experiment at the stream outlets, though it varied little, ranging from 16 to 17 °C.

Algal assemblages on the tiles were examined microscopically at the end of the experiment. Qualitative examinations were made with live material at 100 and 400× magnification, with and without phase contrast optics. Bacteria and microfauna (e.g. ciliates) were present in the periphyton, but algal cells were visibly the dominant contributors to periphyton biomass, even at the lowest light treatments. Because few soft-bodied algae were observed in the qualitative samples, quantitative analysis was limited to counting diatoms at 1000× in samples cleared with hydrogen peroxide and mounted in Naphrax. Cell volumes for individual species were calculated with geometric formulae from Hillebrand *et al.* (1999) or with simple three-dimensional models, using dimensions measured on at least 10 individual cells for each major species.

Data analysis

Since phosphorus was applied on a whole-stream basis and only five streams were available for this study, there was no replication of the five phosphorus treatments. This necessarily limited the statistical analysis of phosphorus effects to correlation/regression analysis. Light treatments were replicated in that the full range of treatments was applied in each of the five streams. However, light treatment effects were

analysed by correlation/regression analysis because of the overlap in actual light levels established by the different light treatments (see above). The absence of true replication of the phosphorus treatments prevented the identification of potential phosphorus × light interactions that may have added variance to our results. For these reasons, tests of statistical significance were conservative in this study.

Because individual measurements of periphyton phosphorus content could be associated with individual measurements of light and dry mass, they were used in multivariate linear regression instead of SRP. This enabled the simultaneous examination of light and phosphorus effects on growth and final dry mass that would not have been possible with SRP. However, we recognize that periphyton phosphorus samples taken within a single stream are not completely independent.

Results

Experimental conditions

Significant gradients of both dissolved phosphorus concentration and light intensity resulted from the experimental manipulations. Mean phosphorus concentrations in the streams during the experiment were calculated from data on stock solution usage and recorded discharge rates. These calculations and the three spot measurements indicated that phosphorus concentrations in the streams were close to targeted concentrations (Table 1). Light emitted from the metal

Table 1 Phosphorus and light conditions in the experimental streams

	Streams					
	6	12	25	75	150	Mean
Phosphorus (µg L^{-1})						
Calculated	5	11	22	82	166	
Measured	5 ± 1	12 ± 1	18 ± 6	69 ± 2	125 ± 47	
Light (µmol m^{-2} s^{-1})						
0 screens	45 ± 2	47 ± 2	64 ± 10	68 ± 11	47 ± 4	54
1 screen	48 ± 4	52 ± 5	34 ± 5	47 ± 8	32 ± 3	42
2 screens	29 ± 3	22 ± 2	22 ± 4	23 ± 4	22 ± 3	23
3 screens	18 ± 2	16 ± 2	18 ± 3	20 ± 4	12 ± 1	17

Streams are identified by their phosphorus target concentrations. Values for measured phosphorus (soluble reactive phosphorus) and light (photosynthetically active radiation) are means ± SD. $N = 3$ for phosphorus measurements; $n = 9$ for light measurements.

halide lamps varied from lamp-to-lamp (and therefore from location-to-location), so there was some overlap between treatments. Nonetheless, the light treatments resulted in a strong gradient of light intensity in each stream (Table 1).

Light effects

Periphyton growth was light-limited over the range of irradiances used in this experiment. The dry mass of periphyton at the end of the experiment was highly correlated with light (Fig. 2a) and, although there was more scatter in the relationship, net biomass-specific growth (μ) also was significantly correlated with light (Fig. 2b). The tighter relationship between dry mass and light was due at least in part to the fact that each dry mass measurement made at the end of the experiment could be associated with an irradiance
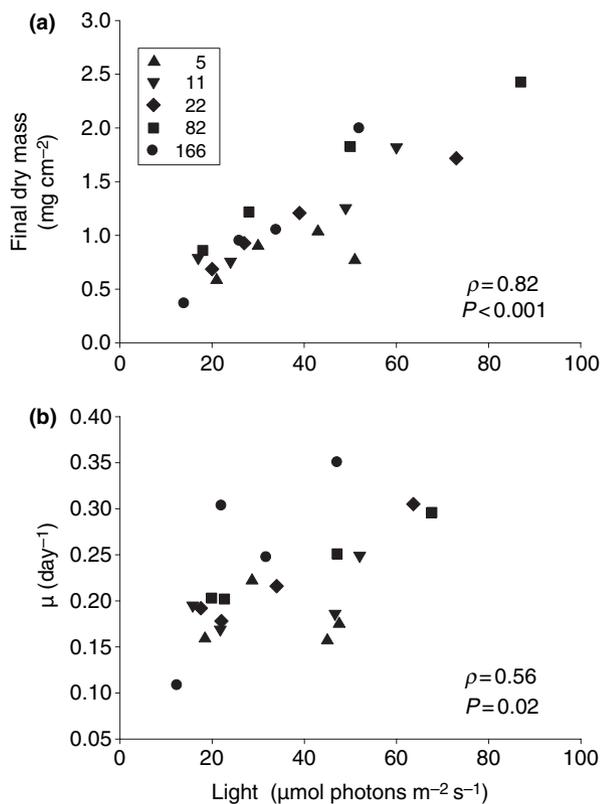
measurement made at the specific location of the final tile sampled, whereas biomass-specific growth rate was necessarily associated with light values that were the means of irradiances measured at nine different locations under each screen.

Phosphorus effects

Net biomass-specific growth was also a function of dissolved phosphorus concentration. Growth rate (averaged over the different light levels in each stream) increased rapidly at low phosphorus concentrations, then began to plateau above 22 $\mu g\ L^{-1}$ SRP (Fig. 3a). The hyperbolic Monod equation fitted the

Fig. 2 Effects of light on final periphyton biomass and net bio-mass-specific growth rate. Numbers in legend refer to the mean experimental SRP calculated over the course of the experiment. Light (photosynthetically active radiation) values in (a) are individual measurements whereas light values in (b) are means of nine individual measurements. Spearman's correlation coefficients (ρ) and associated probabilities are shown.
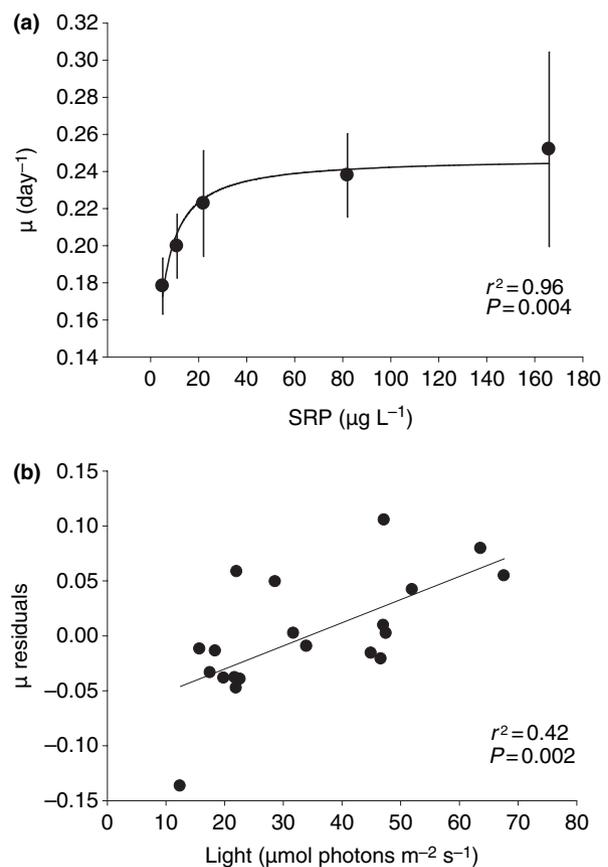

Fig. 3 Effect of dissolved phosphorus on periphyton growth. Dissolved phosphorus is the mean experimental soluble reactive phosphorus (SRP) calculated from total phosphorus dripped into the streams and mean discharge of the streams over the course of the experiment, as in Fig. 2. (a) Symbols represent the mean growth rate for all light treatment levels within each stream; error bars are SE [$n = 4$]. The line is the Monod equation fitted to mean growth rate; $\mu = \mu_{max}\ (SRP)/[(k_u + (SRP)]$, where $\mu_{max} = 0.25$ and $k_u = 2.17$. (b) Linear regression of the residuals from the Monod equation versus light.

mean growth rates very well ($r^2 = 0.96$), but there was considerable variation around the means (note large error bars in Fig. 3a). Much of this variation was due to light effects, as illustrated by the linear regression of residuals from the Monod line versus light (Fig. 3b). The correlation between mean growth rate and SRP was statistically significant ($\rho = 1.00$, $P = 0.02$), despite the limited number of observations ($n = 5$).

Phosphorus content of periphyton

Periphyton phosphorus was related with dissolved phosphorus in a nonlinear fashion (Fig. 4a). There was an initial increase in periphyton phosphorus to a maximum at an SRP concentration of 82 μg L^{-1}, but periphyton phosphorus appeared to decline slightly at the highest SRP concentration of 166 μg L^{-1}. Periphyton phosphorus did not decrease with increasing light for any SRP treatment (Fig. 4b), contrary to the prediction of the Light : Nutrient Hypothesis. Rather, there appeared to be a positive, though weak, effect of light ($\rho = 0.37$, $P = 0.11$). The lowest values of periphyton phosphorus were consistently found in the most shaded periphyton, irrespective of SRP concentration (Fig. 4a). Periphyton phosphorus was highly correlated with specific growth rate (Fig. 4c).

Multivariate analysis

Periphyton dry mass at the end of the experiment was clearly a function of both light and phosphorus content (Fig. 5). Multiple regression confirmed the significant additive effect of phosphorus content as a predictor of final dry mass, with light and phosphorus content together accounting for 81% of the variability in periphyton dry mass (Table 2). Periphyton phosphorus at the end of the experiment was also a significant co-predictor of biomass-specific growth, though the combined effects of both light and phosphorus content accounted for less variability than in the regression of final dry mass (Table 2).

Algal assemblages

Algal assemblages were in turn dominated by large diatoms. Of the 24 species identified, six accounted for >96% of biovolume. Mean ($\pm$SE) relative biovolumes of these six species were: *Melosira varians* Agardh
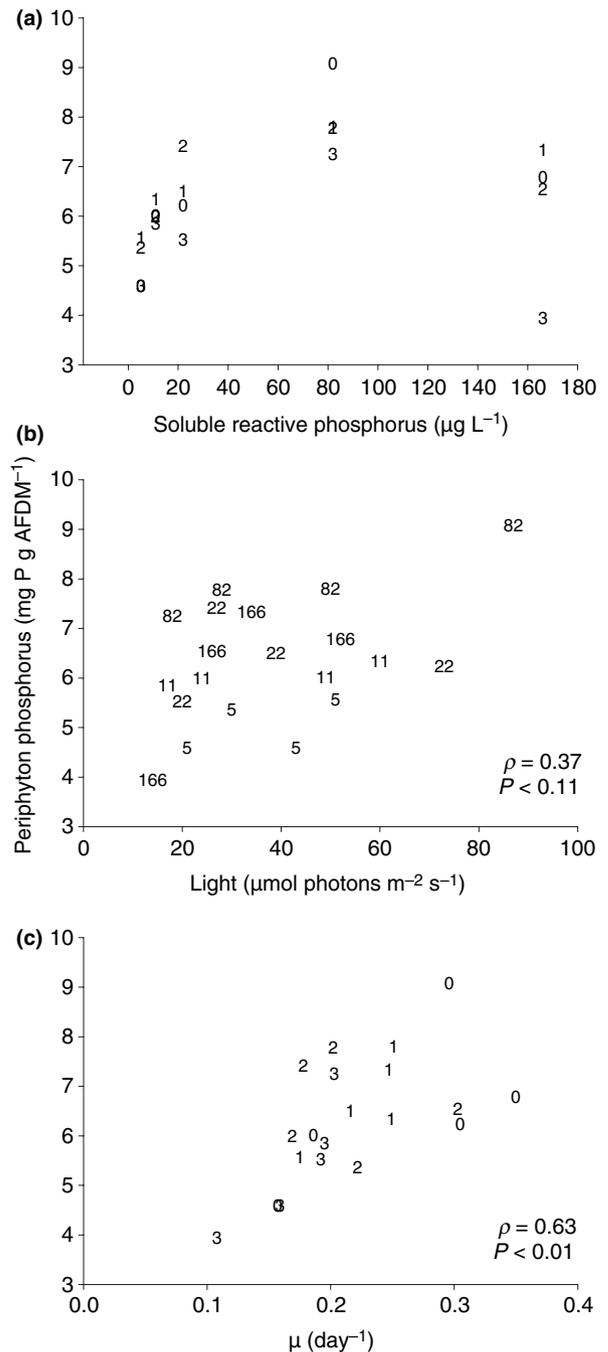

Fig. 4 Periphyton phosphorus as a function of dissolved phosphorus, light and growth rate. Numerical symbols in (a) refer to the level of screening (number of shade screens), so larger numbers represent darker conditions. Numeric symbols in (b) represent the calculated mean concentration of SRP during the entire experiment. The lowest number (5) represents the mean SRP concentration in the stream where no phosphorus was added. Numerical symbols in (c) are the same as in (a).
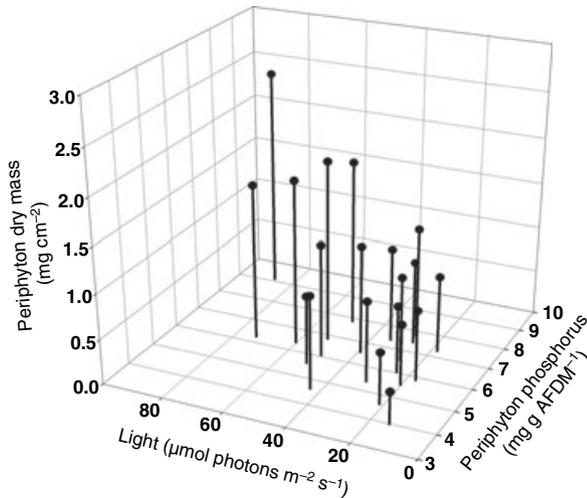
Fig. 5 Periphyton biomass as a function of both light and phosphorus content.

(50% ± 6; *Gomphonema truncatum* Ehrenberg (35% ± 4), *Fragilaria rumpens* Kützing (5.3% ± 0.5), *Meridion circulare* (Greville) Agardh. (3.4% ± 1.2), *Eunotia pectinalis* Rabenhorst (1.5% ± 0.6), and *Achnanthidium minutissima* Kützing (1.0% ± 0.2). Assemblage composition did not appear to be strongly affected by either light or phosphorus treatments; correlations (Spearman's ρ) between relative biovolumes of individual taxa with light or phosphorus concentration were all statistically insignificant ($P > 0.1$), excepting a negative correlation ($\rho = -0.71$, $P < 0.02$) between light and *E. pectinalis*, a minor contributor to total algal biovolume.

Discussion

Colimitation

Three lines of evidence indicate that light and phosphorus colimited algal growth in this study. First, the mean growth rate increased as streamwater phosphorus increased (Fig. 3a). Secondly, residuals from non-linear regression of growth versus streamwater phosphorus were strongly correlated with light (Fig. 3b). Thirdly, both light and periphyton phosphorus content were significant coefficients in the multiple regression analyses of periphyton growth and final dry mass. The key consideration in all these analyses is that the stimulatory effects of phosphorus on periphyton growth occurred at relatively low irradiances.

The irradiances used in this study (<90 μmol photons m^{-2} s^{-1}) were likely to have been subsaturating and growth limiting because: (i) linear regressions of periphyton growth and final dry mass were highly significant; (ii) photosynthesis of most benthic algal assemblages saturates above 100 μmol photons m^{-2} s^{-1} (Hill, 1996) and (iii) detailed analyses of growth versus irradiance relationships for diatom assemblages in the ORNL laboratory streams show that growth rates do not plateau until irradiance exceeds 100 μmol photons m^{-2} s^{-1} (Hill W.R., unpubl. data). Because the stimulatory effects of phosphorus occurred at irradiances below those that approach photosaturation, simultaneous (rather than sequential) limitation of algal growth by light and phosphorus was indicated.

Data supporting the simultaneous limitation of algal growth by light and nutrients are rare. A few studies on phytoplankton species in culture have reported colimitation by light and nutrients (Knoechel & deNoyelles, 1980; Rhee & Gotham, 1981; Fahnenstiel *et al.*, 1984; Healy, 1985) and Fahnenstiel *et al.* (2000) suggested that phytoplankton communities in the Great Lakes are concurrently controlled by both light and phosphorus concentration during spring mixing. Reports of light and nutrients colimiting periphyton growth rates are similarly scarce.

Table 2 Multiple regression of periphyton growth parameters versus light and periphyton phosphorus content

Dependent variable	Regression type	Independent variable(s)	Coefficient ± SE	*P*-value	r^2 (adj.)
Final dry mass	Univariate	Light	0.019 ± 0.003	<0.001	0.67
	Multivariate	Light	0.015 ± 0.003	<0.001	0.81
		P content	0.158 ± 0.041	0.001	
Growth rate	Univariate	Light	0.0021 ± 0.0007	0.009	0.28
	Multivariate	Light	0.0015 ± 0.0007	0.044	0.43
		P content	0.0213 ± 0.0090	0.031	

Greenwood & Rosemond (2005) described unreplicated whole-stream nutrient additions that caused an increase in periphyton growth rate in the shade of trees, and Taulbee, Cooper & Melack (2005) reported increased chlorophyll-*a* on nutrient-diffusing substrata that were also shaded by terrestrial vegetation. Although several stream experiments have failed to find nutrient limitation at low light intensities (e.g. Lowe *et al.*, 1986; Hill & Knight, 1988; Mosisch *et al.*, 2001), the results of this study suggest that light and nutrients can complement each other, with one resource facilitating the acquisition of the other.

Nitrogen is usually identified as the limiting nutrient in the few studies that do report simultaneous limitation of algal growth by nutrients and light (e.g. Taulbee *et al.*, 2005). Ecophysiological research on photoacclimation provides a conceptual basis for the argument that nitrogen availability influences the ability of light-limited algae to acquire scarce photons and thereby increase growth rate at low light supply. Cell quotas of nitrogen are known to be higher in algae growing under low light (Rhee & Gotham, 1981). Such shade adaptation is a consequence of an increased rate of photosynthesis at low irradiance and is linked to an increased efficiency of photon capture. The functional changes occurring in shade adaptation are typically associated with an accelerated synthesis of antenna photopigments (including chlorophyll-*a*) that absorb light. The synthesis of photosynthetic pigments and associated membranes has a relatively high requirement for nitrogen (Geider *et al.*, 1998; Sterner & Elser, 2002). It has recently been suggested that shade adaptation also requires an increased cell quota of phosphorus. Hessen *et al.* (2002) reported that the cell-specific chlorophyll content of *Selenastrum capricornutum* Printz depended on both phosphorus and light, concluding that chlorophyll-*a* synthesis could be directly constrained by phosphorus availability. They hypothesized that the synthesis of chloroplast membranes was limited by the availability of phosphorus for membrane phospholipids.

There was little evidence that the colimiting effect of phosphorus in our study was the result of shade adaptation enabled by the addition of phosphorus. If additional phosphorus was necessary for increasing the synthesis of chlorophyll-*a* or chloroplast membranes, higher cell quotas of phosphorus would be expected at low light. However, periphyton phosphorus content was not negatively correlated with light. If anything, the relationship between light and periphyton phosphorus was positive. We hypothesize that the positive effect of phosphorus on periphyton growth was simply the result of an increased requirement for P-rich ribosomal RNA in faster growing cells, independent of the cause of faster growth. Growth rates and the quantity of ribosomal RNA (and associated phosphorus) are highly correlated in a wide range of organisms (Vrede *et al.*, 2004). The highly significant positive correlation between periphyton growth rate and phosphorus content observed here is consistent with the hypothesis that additional phosphorus was needed for the general protein manufacture required for biosynthesis rather than for the specific manufacture of photon-capture machinery. A *caveat* to this hypothesis is that the relationship between growth and phosphorus content may change in the later stages of development, when slow-growing senescent cells containing moderately high levels of phosphorus become more abundant.

Periphyton phosphorus content

The pattern of periphyton phosphorus content observed in this study is inconsistent with the Light : Nutrient Hypothesis of algal stoichiometry. Light did not have the negative effect on phosphorus content predicted by the hypothesis, even at the lowest phosphorus concentrations. It is unclear why this should be the case, though it should be noted that a negative effect of light on periphyton phosphorus content has not been found in streams and demonstrated only inconsistently in lentic habitats (Frost & Elser, 2002; Hillebrand *et al.*, 2004). Benthic algae in streams may be less susceptible to the diluting effects of excess photosynthate at higher light intensities than are lentic algae (benthic and planktonic) because algae are less likely to be nutrient-limited in flowing waters (Borchardt, 1996). Alternatively, the crowded matrix of highly productive periphyton communities may result in the depletion of inorganic carbon [e.g. $CO_2(aq)$] within the community matrix (Hill & Middleton, 2006), limiting the rate of photosynthesis and, therefore, the amount of carbon available to dilute phosphorus and other elements within algal cells. The concentration of $CO_2(aq)$ should logically affect C : N : P ratios in algae, but the experimental support for increased nutrient content at higher $CO_2(aq)$ concentrations is

mixed (Sterner & Elser, 2002; Urabe, Togari & Elser, 2003; Hessen *et al.*, 2004). In any case, the evidence so far does not indicate that an 'excess' of light will seriously affect the food quality of stream algae and the growth of lotic grazers, as has been suggested for lakes (e.g. Urabe *et al.*, 2002).

In contrast to the minor effect of light, the effect of phosphorus availability on periphyton phosphorus content generally fit quantitative expectations. Nutrient uptake in periphyton is expected to follow Michaelis–Menten kinetics and fit a rectangular hyperbola function with dissolved nutrients (e.g. Rhee, 1973; Borchardt, 1996). The phosphorus content of periphyton in this study increased nonlinearly with SRP concentration, reaching a maximum at 82 μg L^{-1} SRP. Phosphorus content did appear to decrease at the highest SRP concentration, but it was unclear whether this was a result of a true response to the high concentration, the result of uncontrolled stream-to-stream variability (there were no true replicates for SRP concentration in this study), or the influence of growth rate. Much of the apparent decrease at the highest concentration was due to a single observation taken from the most highly shaded and slowest growing periphyton in this study. The phosphorus content of this observation may have been a stronger reflection of the link between phosphorus content and growth rate (as discussed above) than an inhibition response to high phosphorus concentrations.

Components of periphyton other than algal cells undoubtedly contributed to periphyton phosphorus. If these components were abundant and their stoichiometry differed from algae, their response to light or phosphorus could have conceivably affected our results. For example, bacterial growth may have been stimulated by an increase in the dissolved carbon excreted by algal cells at higher irradiance and, if bacterial phosphorus content is higher than that of algae (Sterner & Elser, 2002), bacterial growth could be responsible for increasing periphyton phosphorus with light. We think the effect of non-algal components on periphyton phosphorus was small because microscopic examination of live periphyton did not reveal large biovolumes of bacteria or microfauna relative with the biovolume of diatoms. However, we are reluctant to dismiss non-algal components as potential influences on periphyton phosphorus.

Implications for nutrient standards

The results of this study have important applications. The development of nutrient standards to protect streams from eutrophication is hampered by a scarcity of experimental data. A number of observational studies and meta-analyses have attempted to quantify the relationship between nutrient concentrations and the growth of periphyton in streams, but these studies have been plagued by tremendous variability in periphyton biomass (e.g. Dodds, Smith & Lohman, 2002), at least partly because multiple factors determine the accrual of periphyton biomass. Determining concentration thresholds with such highly variable data sets is difficult at best. Our study is one of the few to apply experimental gradients of phosphorus in seeking to quantify the relationship between dissolved phosphorus and periphyton growth (but see Horner, Welch & Veenstra, 1983; Bothwell, 1989; Rier & Stevenson, 2006), and it is the only one to apply simultaneous gradients of both light and phosphorus.

The relationship between periphyton growth and phosphorus concentration in this study suggests that nutrient criteria allowing $\geq$25 μg L^{-1} of bioavailable phosphorus (e.g. SRP) will do little to preclude eutrophication in streams. Growth rates appeared to be nearing a plateau (saturation) at our target phosphorus concentration of 25 μg L^{-1} (= calculated mean concentration of 22 μg L^{-1}), indicating that phosphorus concentrations would need to be substantially reduced below 25 μg L^{-1} to effect a significant decrease in periphyton accrual rate. A *caveat* to this conclusion is that it is based on the response of an algal assemblage composed of a limited set of diatoms. It is unclear how representative the nutrient response of assemblages dominated by the diatoms *M. varians* and *G. truncatum* is of stream assemblages in general, but both taxa are widely distributed in streams, and *M. varians* is associated with eutrophic conditions (Lowe, 1974). In addition, the 25 μg L^{-1} threshold level suggested here is similar to threshold levels reported by researchers whose experimental algal assemblages differed from ours (Horner *et al.*, 1983; Bothwell, 1989; Rier & Stevenson, 2006).

Phosphorus effects on algal growth clearly need to be considered within the context of light limitation, especially in streams, where light regimes vary dramatically on both seasonal and spatial scales (Hill, 1996). Light accounted for major proportions of the

variance in growth and final biomass of periphyton in this study, with phosphorus assuming a secondary role. These results were of course affected by the particular supplies of light and phosphorus available, and the relative effect of the two factors could have been different if lower phosphorus concentrations had been employed. Nonetheless, the lowest concentration in this study, 5 μg L^{-1}, is lower than the mean SRP of the most pristine U.S.A. streams sampled by Omernik (1977) and is well below the 25 μg L^{-1} total phosphorus concentration suggested by Dodds, Jones & Welch (1998) as the upper boundary of oligotrophy for streams. Despite the secondary role phosphorus may play under highly shaded conditions, our results show that phosphorus can have an effect on periphyton growth even at subsaturating irradiances. We suggest that nutrient standards be applied to streams regardless of their light regimes.

Acknowledgments

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