

## Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications for developing nutrient criteria

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### Abstract

Responses of stream algal biomass to nutrient enrichment were studied in two regions where differences in hydrologic variability cause great differences in herbivory. Around northwestern Kentucky (KY) hydrologic variability constrains invertebrate biomass and their effects on algae, but hydrologic stability in Michigan (MI) streams permits accrual of high herbivore densities and herbivory of benthic algae. Multiple indicators of algal biomass and nutrient availability were measured in 104 streams with repeated sampling at each site over a 2-month period. Many measures of algal biomass and nutrient availability were positively correlated in both regions, however the amount of variation explained varied with measures of biomass and nutrient concentration and with region. Indicators of diatom biomass were higher in KY than MI, but were not related to nutrient concentrations in either region. Chl *a* and % area of substratum covered by *Cladophora* were positively correlated to nutrient concentrations in both regions. *Cladophora* responded significantly more to nutrients in MI than KY. Total phosphorus (TP) and total nitrogen (TN) explained similar amounts of variation in algal biomass, and not significantly more variation in biomass than dissolved nutrient concentrations. Low N:P ratios in the benthic algae indicated N as well as P may be limiting their accrual. Most observed responses in benthic algal biomass occurred in nutrient concentrations between 10 and 30  $\mu\text{g TP l}^{-1}$  and between 400 and 1000  $\mu\text{g TN l}^{-1}$ .

### Introduction

Problems with high algal biomass due to nutrient enrichment in streams and other habitats has been the subject of considerable concern (Carpenter et al., 1998; Smith et al., 1999) and have led to efforts by the US Environmental Protection Agency to develop nutrient criteria that prevent nuisance growths of algae (USEPA, 1999). Many

studies have documented effects of nutrients on algae (Bourassa & Cattaneo, 1998; Dodds et al., 1998; Biggs, 2000), but relationships in regional studies are seldom precise or accurate enough to predict benefits of specific nutrient regulations for streams throughout a region. In addition, many factors could affect algal–nutrient relationships in streams within a region and among regions with different climate, geology, water chemistry,

and hydrology (Biggs et al., 1990, 1998; Stevenson, 1997b). Understanding differences in algal–nutrient relationships between very different regions would help establish the range of possibilities that can be expected and the factors that regulate that range.

Disturbance regime has been hypothesized to be a major factor regulating between-region differences in algal–nutrient relationships in streams. High current velocities associated with elevated discharge following rains scour algae directly or indirectly by tumbling substrata (Power & Stewart, 1987; Biggs & Thomsen, 1995). Disturbance regimes with low intensity or high predictability (Poff & Ward, 1989) support development of high grazer densities that constrain algal growths (Wootton et al., 1996; Riseng et al., 2004). Alternatively, high intensity and frequency disturbance regimes could constrain algal growth (Biggs, 1995; Clausen & Biggs, 1997). Shading and regional factors such as water chemistry and temperature may also affect the ability of large growths to occur by limiting photosynthesis or colonization of sites by species capable of producing nuisance growths.

Lack of precision in relationships between algal biomass and nutrient concentrations may also be due to variability in estimates of algal biomass and nutrient concentrations in streams. Algal biomass varies greatly during colonization after storm disturbances (Fisher et al., 1982). Patchy growths of macroalgae throughout a reach and even a riffle make assessment of algal biomass difficult when sampling. The phyla and growth forms of algae (i.e., functional group) vary seasonally from diatoms to filamentous green and blue-green algae (Blum, 1957; Hynes, 1970). Each functional group has the potential to develop very different biomasses. Nutrient concentrations vary: diurnally with microbial metabolism; daily with weather-related hydrologic factors and with increasing biomass and nutrient uptake during periphyton community development after storms; and seasonally with human activities and metabolism of terrestrial vegetation in watersheds (Meyer et al., 1988; Kim et al., 1992). A sampling approach with extensive and repeated sampling during the period of algal community development should reduce error variance in algal–nutrient relationships by

accounting for spatial and temporal variability in nutrient concentrations.

The objectives of this study were to relate benthic and suspended algal biomass to nutrient concentrations in streams of two regions with very different disturbance regimes and where very different relationships were expected. We expected that algal–nutrient relationships would vary with type of algae, the nutrient parameter, and the region studied such that more precise relationships could be quantified if these factors were accounted for. Changes in algal biomass along nutrient gradients were studied for thresholds and additional justification for establishing nutrient criteria to prevent nuisance growths of benthic algae.

## Methods

### *Field methods*

Benthic algal biomass was assessed in 104 streams in two regions of the north-central region in the United States (Fig. 1). These regions were chosen because of observed differences in grazing effects on benthic algae that have been related to disturbance regimes in the streams (Riseng et al., 2004). Benthic invertebrate biomass was significantly lower in streams of the Kentucky–Indiana region (KY) than Michigan (MI) (Riseng et al., 2004), *t*-test,  $p < 0.001$ ). Biomass of invertebrates was usually less than  $2 \text{ g m}^{-2}$  in the KY and greater than  $3 \text{ g m}^{-2}$  in MI. Invertebrates assemblages were dominated by mayflies in the KY region and by caddisflies in MI.

The KY region included 46 streams of the unglaciated Knobs region of northwestern Kentucky and southeastern Indiana, which is typified by rapid runoff due to steep topography and the underlying limestone bedrock (Burroughs, 1926; McGrain, 1983). Fifty-eight streams were located in the glaciated region of Michigan's Lower Peninsula (MI region), where precipitation slowly percolates to the groundwater and thereby, contributes to stable, consistent discharge rates (Wiley et al., 1997). Streams were chosen to represent a wide range of nutrient conditions, which were predicted based on land use/land cover types. Land use/land cover ranged from relatively pristine

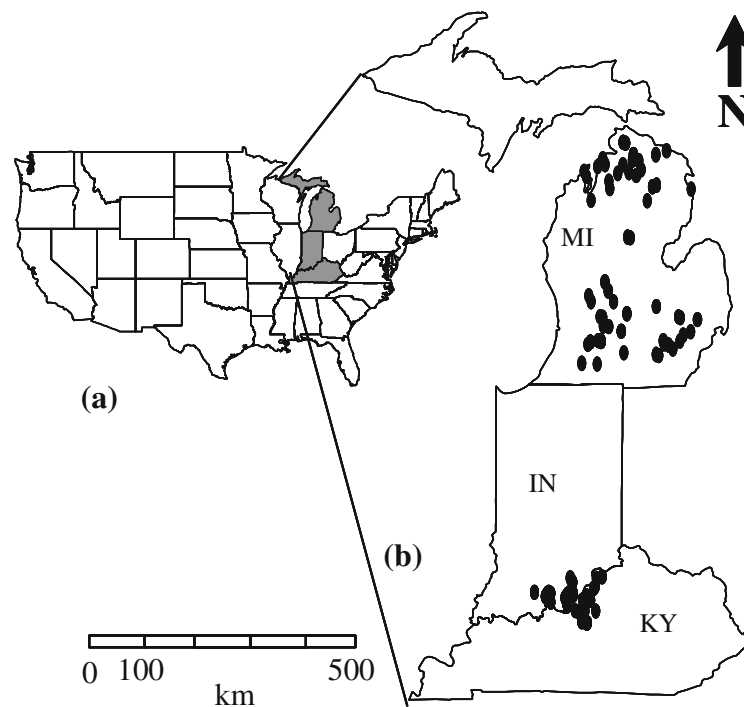


Figure 1. Location of streams sampled in northwestern Kentucky (KY) and southeastern Indiana (IN) and in Michigan (MI, USA).

forested and wetland areas to urban streams. Most stream sites flowed through a mixture of agricultural and forested land. Stream size ranged from 1st to 4th order (Strahler, 1952) with most streams being 2nd and 3rd order.

Sampling was conducted during 2-month sampling periods for each stream, May and June in KY and July and August in MI, during both 1996 and 1997. The sampling periods were chosen to control for temporal differences in water temperature, stream flow, and observed periods of *Cladophora* growth caused by latitudinal and hydrologic differences between the regions. Flow and *Cladophora* blooms were commonly gone in many KY streams by early summer. We restricted our sampling to riffles to minimize habitat variation among study sites and to concentrate on areas where nuisance algal proliferations tend to be greatest.

We conducted 145 assessments of algal biomass, nutrient concentrations, and habitat features in 104 streams, with 74 assessments in 1996 and 71 in 1997. Forty-one of the stream sites sampled in 1996 were re-sampled in 1997 because of the limited number of sites in the regions targeted. Eight

pairs of sites (four from each region) were from the same stream in a watershed, but the lower sites were always at least 5 river kilometers downstream of the upper site. We assumed all 145 assessments were independent because flood flows in the same stream among years are commonly assumed to be independent for hydrologic and population time-series analysis in rivers (Cobb et al., 1992; Gordon, 1995; Kohler & Wiley, 1997). Riseng et al. (2004) found significant inter-annual differences in grazer and filter-feeder populations at our revisited sites (paired *t*-test,  $p < 0.05$ ) and that peak discharge and benthic chlorophyll levels were not correlated among sites between years.

Each assessment involved 8–9 visits to KY streams and  $\leq 4$  visits to MI streams during a 2-month sampling period. A higher sampling frequency was used in KY versus MI because hydrologic variability was less and sites were much farther apart in MI than KY. During these weekly or biweekly visits, discharge was assessed with a Marsh–McBirney current meter; pH and conductivity were determined with a YSI meter (YSI Incorporated, Yellow Springs, Ohio, USA); water temperature was determined with a thermometer;

and canopy cover was assessed with a spherical canopy densiometer. Samples for nutrient and chloride assessments were collected in 2 125-ml acid-rinsed polyethylene bottles. Water in one sample was filtered in the field through 0.45- $\mu\text{m}$  pore-size filters to measure dissolved nutrients. Nutrient samples were stored on ice until returning to the lab where they were frozen until analysis.

Rapid periphyton surveys were also conducted during the weekly or biweekly visits to a site. This protocol was used for assessing algal biomass visually at larger spatial scales than practical when scraping algae on rocks and for distinguishing biomass composed of major phyla and growth forms of algae. A viewing bucket was constructed with the bottom cut out of a plastic tub and replaced with a clear plastic circle with a water-tight seal. Use of a viewing bucket reduced glare from sunlight on the water surface and improved visibility of the bottom in these streams with relatively high light levels. A 50-point grid was marked on the bottom plastic circle to facilitate characterizing the percent of stream bottom in different conditions. Visual assessments were taken at nine locations throughout the targeted riffle at each site. Each assessment involved submerging the bottom of the viewing bucket in the water and characterizing: (1) % of the bottom covered by macroalgae (e.g., *Cladophora*); (2) the average cover of suitable substrata (>2 cm diameter); and (3) thickness of the each different type of microalgae (e.g., diatoms and blue-green algae). Thickness of microalgae was characterized with a ranking system in which: 0 indicated the rock felt rough and had very little or no periphyton; 0.5 indicated the rock felt slippery, but no periphyton could be seen; 1 indicated periphyton was visible in a very thin biofilm; 2 indicated periphyton with a thickness > thin biofilm but  $\leq 1$  mm; 3 indicated periphyton with a thickness >1 mm but  $\leq 5$  mm; 4 indicated periphyton with a thickness >5 mm but  $\leq 2$  cm; and 5 indicated periphyton >2 cm thick. The average diatom thickness rank and chl *a* (method described later) were determined separately on 50 rocks to assess the reliability of visual assessments of diatom biomass.

Algae on rocks were sampled during the last two weeks of the 2-month sampling period and more than 5 days after a moderate storm event. Benthic algae were sampled using a spoon and

toothbrush from the tops of 15 rocks randomly selected in the riffle at each site. Subsamples for different assays were separated in the field and stored on ice for chl *a* and mat chemistry analysis and preserved with M3 for AFDM assays. Estimates of rock surface areas from which algae were scraped were made in the field by measuring the upper surface of rocks.

Water samples were analyzed for Cl,  $\text{NO}_3 + \text{NO}_2$  ( $\text{NO}_x$ ), and  $\text{NH}_4$  using a Skalar<sup>®</sup> auto-analyzer, for soluble reactive P (SRP) using a Hitachi<sup>®</sup> U-2001 spectrophotometer, and for alkalinity according to standard methods (APHA, 1998). To determine TP and TN concentrations, particulate matter in water samples was oxidized with persulfate and analyzed for SRP and  $\text{NO}_x$  (D'Elia et al., 1977; APHA, 1998). Mat chemistries were determined by subsampling portions of mats and analyzing the samples for TN and TP as described for water samples. Mat chemistries were calculated as the proportion of N and P in periphyton samples (including algae, meiofauna, bacteria, detritus, and silt) by dividing the mass of N and P in samples by the AFDM. AFDM was measured after drying samples in aluminum pans and ashing at 500 °C (APHA, 1998). Chlorophyll *a* was extracted from algal samples with 90% buffered acetone and measured spectrophotometrically (APHA, 1998).

#### *Data analysis*

Simple correlation, linear and non-linear regression, and multivariate regression approaches were used to determine relationships between algal biomass and nutrient concentrations in streams of the two regions. All statistical analyses were calculated using SYSTAT<sup>®</sup> version 10 (Wilkinson, 1990). Variables with skewed distributions were natural-log transformed to produce more even and normal distributions. Descriptive statistics and *t*-tests were used to determine whether significant differences in environmental conditions occurred between regions. Covariance matrices for nutrient concentrations and algal biomass were calculated independently to evaluate differences among regions. Correlations between the three algal biomass attributes (benthic chl *a*, diatom rank, average *Cladophora* cover during the

sampling period) and average nutrient concentrations at sites were calculated independently for each region and for both regions combined to determine the importance of region for explaining variation in algal biomass–nutrient relationships. A fourth algal biomass attribute, maximum *Cladophora* cover at a site during a sampling period, was included in assessment of the probability of different average and maximum *Cladophora* covers occurring in streams with different nutrient concentrations.

Linear and non-linear regression analyses were used to test the hypothesis that non-linear models explained significantly more variation in algal biomass–nutrient relationships than linear models. The Monod equation was modified for the non-linear model,

$$B = B_{\max} * (S / (S + K_s))$$

where biomass ( $B$ ) are predicted as a function of the maximum biomass possible ( $B_{\max}$ ), nutrient concentrations ( $S$ ), and a nutrient concentration at which half of  $B_{\max}$  would occur ( $K_s$ ). The Monod model was selected *a priori* because we hypothesized that algal biomass would increase rapidly and then saturate with progressive increases in nutrient concentrations in the same way as growth rates. Stepwise multiple regression was used to determine whether TN and TP explained more variation in algal biomass than either nutrient alone or whether other environmental factors such as canopy cover accounted for unexplained variation in biomass–nutrient relationships. TN and TP were used in detailed analyses because these parameters are recommended for nutrient criteria development (USEPA, 1999) and they indicate nutrient availability as well or better than other parameters (Dodds, 2003). The Z-test (Zar, 1974) was used to compare the correlations among analyses and to determine whether differences in the amount of variance explained were statistically significant. Difference in algal responses to nutrients between regions was determined by direct comparison of regression coefficients with standard errors of coefficients and  $t$ -statistics (Zar, 1974). Analysis of covariance was not used to compare relationships between regions because error variances could theoretically be very different in the two regions.

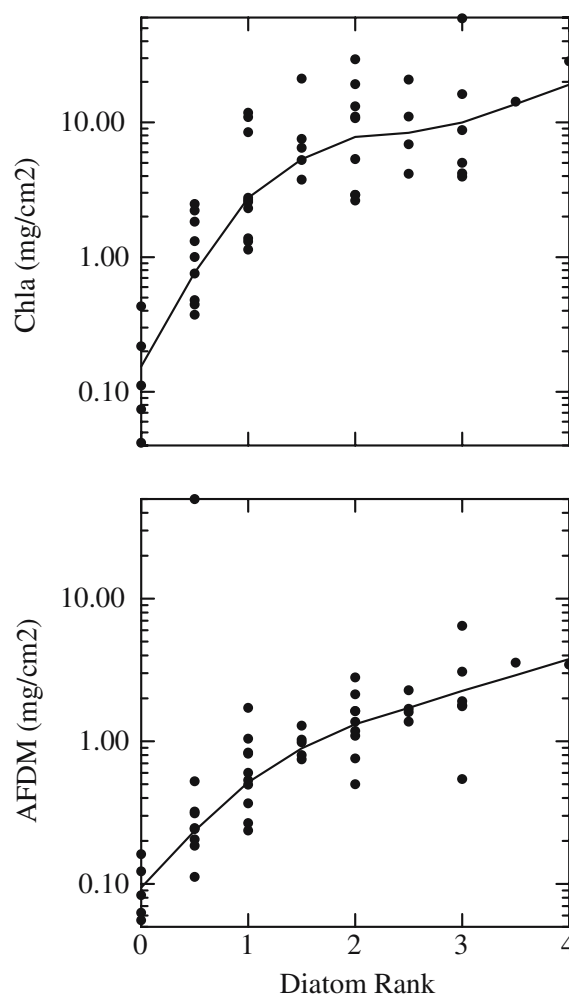


Figure 2. Relationships between chl *a* and AFDM and diatom rank determined by assessment of individual rocks to assess the precision of visual assessments. Lines on figures produced by loess smoothing (Wilkinson, 1990).

## Results

### *Accuracy of rapid periphyton survey*

Estimates of diatom biomass using thickness rank explained over 58 and 46% of the variation in diatom biomass estimated, respectively, with ln-transformed chl *a* and ln-transformed AFDM of periphyton on rocks (Fig. 2). The non-linear pattern in log-transformed chl *a* with increasing diatom rank increased variance explained to 71% by using polynomial regression. The non-linear pattern in AFDM with increasing diatom rank

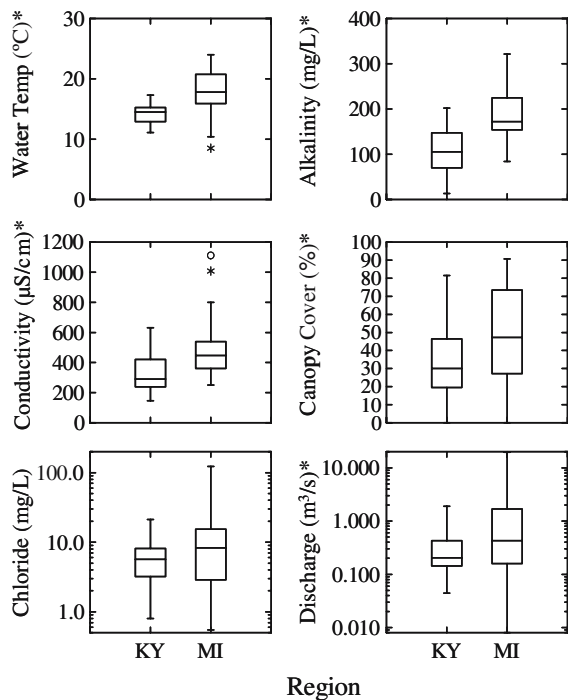


Figure 3. Comparison of non-nutrient abiotic factors between KY and MI. Asterisks indicate mean values were different among ecoregions ( $t$ -test,  $p < 0.005$ ).

was not statistically significant and explained little additional variation compared to the linear model.

#### Comparison of stream conditions between KY and MI

Averages of almost all non-nutrient parameters, except Cl, were significantly different between regions ( $t$ -tests,  $p < 0.05$ ), but ranges of parameters overlapped greatly (Fig. 3). Results indicated that our study streams were moderately buffered with alkalinity averaging 109 and 187 mg l<sup>-1</sup> and pH 8.04 and 7.94 in the KY and MI, respectively. Conductivity was lower in KY than MI. Average water temperatures during the sampling periods were lower in KY than MI (14.2 and 18.0 °C, respectively), streams were smaller in KY than MI (discharge was 0.32 and 1.52 m<sup>3</sup> s<sup>-1</sup>, respectively), but ranges in both temperature and discharge overlapped in the two regions. Ranges in canopy cover (0–>80% cover) and chloride concentrations (0.5–123.5 mg l<sup>-1</sup>) also overlapped greatly between the two regions.

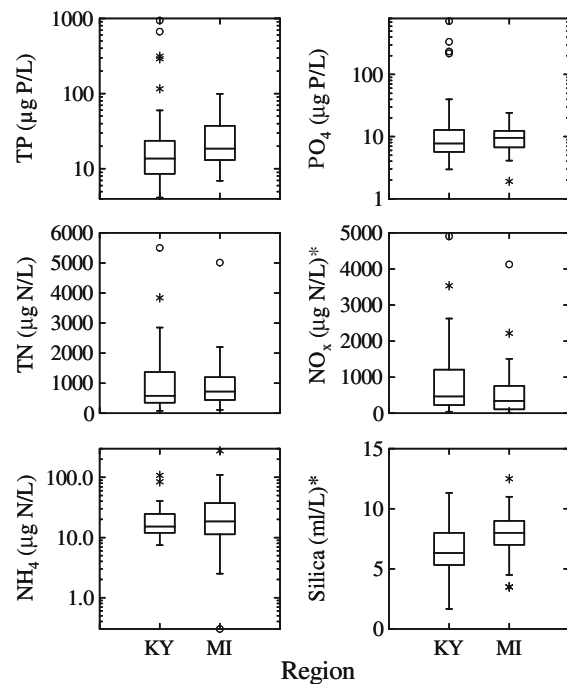


Figure 4. Comparison of nutrient concentrations between KY and MI. Asterisks indicate mean values were different among ecoregions ( $t$ -test,  $p < 0.005$ ).

Average concentrations of TP, SRP, TN and NH<sub>4</sub> and ratios of N:P in water and P:AFDM did not differ between regions and wide ranges in nutrient conditions were observed (Fig. 4). However, NO<sub>x</sub>, Si, mat N:P ratios, and mat N:AFDM ratios differed between regions, even though their ranges overlapped greatly. Many observations of SRP and TP concentrations were less than 10 µg P l<sup>-1</sup> and some were greater than 100 µg l<sup>-1</sup>. SRP averaged 62% of TP in KY and only 36% of TP of MI. All but four TP concentrations in KY were less than 100 µg l<sup>-1</sup>. Figures illustrating algal response to TP were limited to less than 100 to optimize use of space and more clearly illustrate changes in algal biomass with TP. Silica concentrations ranged from 1.7 to 12.5 mg l<sup>-1</sup> with averages of 6.5 and 8.0 in the two regions. Molar N:P ratios averaged 103 and 84 in the water and 11.3 and 9.2 in periphyton in KY and MI, respectively. NO<sub>x</sub> and TN concentrations were often less than 50 and 100 µg N l<sup>-1</sup> respectively, and as high as 5497 µg TN l<sup>-1</sup>. NO<sub>x</sub> was 60 and 83% of TN in the KY and MI, respectively. NH<sub>4</sub>, ranging from less than 5–277 µg N l<sup>-1</sup>, was less than 5% of TN in both regions.

### Relations between nutrients and environmental factors

Nutrient concentrations were highly correlated with each other, but were not highly correlated with alkalinity, canopy cover, and discharge. Pearson correlations among nutrient concentrations were higher among KY than MI streams, with all  $r$  being greater than 0.6 in KY and less than 50% of  $r$  being greater than 0.6 in MI (Table 1). Conductivity and Cl were correlated to nutrients in the MI and KY, but pH was correlated significantly with nutrient concentrations only in KY streams.

Even though  $\ln(\text{TP})$  and  $\ln(\text{TN})$  in streams were correlated with each other in KY and MI ( $r=0.69$  and  $r=0.62$ , respectively), many high N sites had low P concentrations. However, low N sites with high P were absent from the possible combinations of nutrient conditions in both regions. SRP and TP were more highly correlated in KY than MI (Z-test,  $p < 0.05$ ).  $\text{NO}_x$  was highly correlated with TN in both regions and more highly correlated with TN than  $\text{NH}_4$ .

### Determining algal biomass relations with nutrients

Variability in most measures of algal biomass was great in both regions (Fig. 5). Benthic chl  $a$  varied from  $<0.1 \mu\text{g cm}^{-2}$  to  $>40 \mu\text{g cm}^{-2}$ . Average diatom rank at a site varied from less than 0.5 to about 2.0. Average and maximum *Cladophora* cover at a site varied from 0 to 80%. Benthic chl  $a$  and diatom rank were significantly greater in KY (averaging  $13.0 \mu\text{g chl } a \text{ cm}^{-2}$  and thickness rank = 0.92) than MI (averaging  $4.1 \mu\text{g chl } a \text{ cm}^{-2}$  and rank = 0.55).

Correlations between all measures of N or P concentrations and measures of algal biomass were positive (Table 2) and more than half were statistically significant ( $p < 0.05$ , without Bonferroni correction). Diatom rank was not significantly correlated to nutrient concentration in either region, except for  $\text{NH}_4$ . Diatom abundances ranged widely from low to high ranks at all nutrient concentrations in KY, but ranged little in MI streams unless nutrient concentrations were really high (Fig. 4).

Correlations between nutrients and algal attributes were higher for specific regions than when

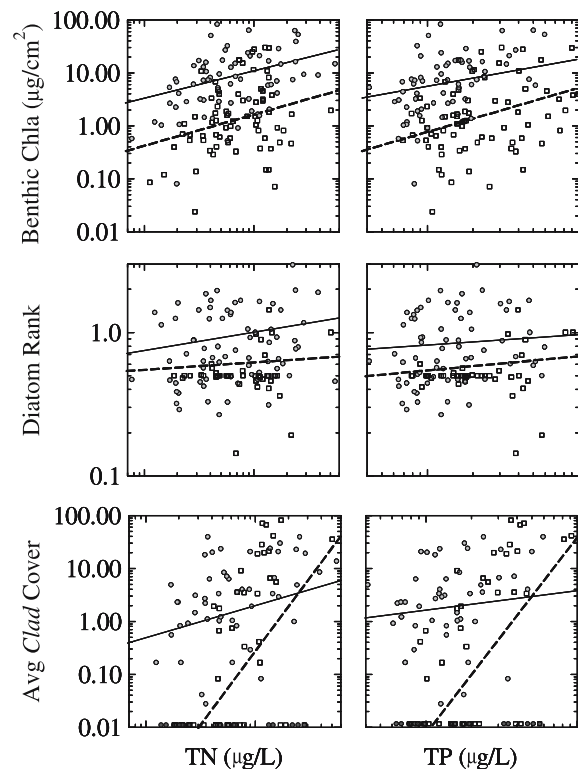


Figure 5. Individual relationships between benthic chl  $a$ , diatom thickness rank, and average *Cladophora* cover and average total nitrogen (TN) and total phosphorus (TP) concentrations in streams in two regions, the Knobs (shaded circles) and the glaciated region (squares). The lines indicate the best fit of linear regression for streams in the Knobs (solid line) and in the glaciated region (dashed line). Statistical significance of the relationships can be found in Table 4.

data from both regions were combined (Table 2), especially for the most highly correlated algal attributes. For example, correlations between *Cladophora* cover and total nutrients averaged 0.354 when data for both regions were combined; they were significantly less (Z-test,  $p < 0.05$ ) than the average  $r=0.608$  in MI where *Cladophora* cover was most highly correlated with TP and TN. Benthic chl  $a$  was better correlated to all nutrients in KY streams than when streams from MI were included in calculations.

$\text{NO}_3$ , SRP, TN, and TP were similarly correlated with measures of algal biomass, except for low correlations between SRP and *Cladophora* cover in MI. Correlations tended to be higher between measures of algal biomass and  $\text{NH}_4$  than other nutrient concentrations. Algal biomass

Table 1. Correlations among water chemistry parameters in streams of the Knobs and glaciated region

	pH	Alk	CanCov	Cond	Cl	NO <sub>x</sub>	NH <sub>4</sub> <sup>+</sup>	TN	SRP	Region
Alkalinity	<b>0.568</b>									Knobs
Canopy cover	<b>0.368</b>	0.12								Knobs
Conductivity	<b>0.515</b>	<b>0.87</b>	-0.007							Knobs
Chloride	<b>0.278</b>	<b>0.279</b>	0.120	<b>0.456</b>						Knobs
NO <sub>3</sub>	<b>0.235</b>	0.150	0.203	<b>0.299</b>	<b>0.758</b>					Knobs
NH <sub>4</sub>	<b>0.353</b>	0.185	<b>0.336</b>	<b>0.339</b>	<b>0.675</b>	<b>0.602</b>				Knobs
TN	<b>0.272</b>	0.205	0.218	<b>0.346</b>	<b>0.786</b>	<b>0.984</b>	<b>0.663</b>			Knobs
SRP	<b>0.407</b>	0.158	<b>0.308</b>	<b>0.348</b>	<b>0.582</b>	<b>0.600</b>	<b>0.733</b>	<b>0.639</b>		Knobs
TP	<b>0.418</b>	<b>0.248</b>	<b>0.255</b>	<b>0.421</b>	<b>0.649</b>	<b>0.645</b>	<b>0.749</b>	<b>0.694</b>	<b>0.960</b>	Knobs
Alkalinity	-0.039									Glaciated
Canopy cover	0.081	0.054								Glaciated
Conductivity	<b>0.269</b>	<b>0.331</b>	0.111							Glaciated
Chloride	<b>0.243</b>	0.082	0.099	<b>0.814</b>						Glaciated
NO <sub>3</sub>	0.093	0.087	0.013	<b>0.528</b>	<b>0.460</b>					Glaciated
NH <sub>4</sub>	0.168	0.088	0.083	<b>0.649</b>	<b>0.662</b>	<b>0.281</b>				Glaciated
TN	0.204	0.014	-0.081	<b>0.601</b>	<b>0.554</b>	<b>0.852</b>	<b>0.435</b>			Glaciated
SRP	-0.029	<b>0.383</b>	0.029	<b>0.514</b>	<b>0.455</b>	<b>0.284</b>	<b>0.616</b>	<b>0.333</b>		Glaciated
TP	<b>0.308</b>	0.010	0.107	<b>0.644</b>	<b>0.588</b>	<b>0.465</b>	<b>0.659</b>	<b>0.616</b>	<b>0.482</b>	Glaciated

correlations with total nutrients were about the same as with dissolved nutrients. Silica concentrations were negatively correlated with benthic chl *a* and *Cladophora* cover in KY and when data for both regions were combined.

Multivariate and non-linear models did not explain significantly more variation in algal biomass than univariate linear models using TN and TP. If  $r^2$  of multivariate and non-linear models were greater than for univariate linear models, then they were not more than 20% greater.

Only 4 of 36 correlations were statistically significant ( $p < 0.05$ ) between algal biomass measures and either water column N:P ratios or periphyton P/AFDM, N/AFDM, or N:P ratios (Table 3). Significantly positive correlations were observed between chl *a* and N/AFDM in MI and both regions together, between diatom rank and N/AFDM in both regions together, and between chl *a* and mat N:P ratio in MI streams. Water column N:P ratios were not related to algal biomass in either region.

Table 2. Correlations between measures of benthic algal biomass and nutrient indicators independently in streams of the Knobs and glaciated region and jointly with data from both regions

Region	Biomass variable	ln(TN)	ln(TP)	ln(NO <sub>x</sub> )	ln(NH <sub>4</sub> )	ln(SRP)	ln(Si)
Knobs	ln (ben chl <i>a</i> )	<b>0.433</b>	<b>0.416</b>	<b>0.399</b>	<b>0.521</b>	<b>0.369</b>	<b>-0.348</b>
Knobs	ln (dia rank)	0.177	0.124	0.141	<b>0.336</b>	0.136	0.116
Knobs	ln (Clad cov)	<b>0.289</b>	0.224	<b>0.267</b>	0.200	0.176	<b>-0.379</b>
Glaciated	ln (ben chl <i>a</i> )	<b>0.298</b>	<b>0.320</b>	0.164	<b>0.528</b>	<b>0.436</b>	0.176
Glaciated	ln (dia rank)	0.223	0.298	0.167	<b>0.471</b>	0.142	-0.059
Glaciated	ln (Clad cov)	<b>0.548</b>	<b>0.668</b>	<b>0.469</b>	<b>0.523</b>	0.129	0.048
Both	ln (ben chl <i>a</i> )	<b>0.269</b>	<b>0.229</b>	<b>0.313</b>	<b>0.393</b>	<b>0.318</b>	<b>-0.263</b>
Both	ln (dia rank)	0.126	0.104	0.181	<b>0.280</b>	0.139	-0.065
Both	ln (Clad cov)	<b>0.368</b>	<b>0.340</b>	<b>0.355</b>	<b>0.361</b>	0.145	<b>-0.217</b>

Correlation coefficients ( $r$ ) in bold indicate statistical significance with  $p < 0.05$ .



Table 3. Correlations between measures benthic algal biomass, nutrient ratios in the water, mat nutrient concentrations and ratios, and other stream chemistry and habitat parameters (CanCov, canopy cover; Cl, chloride; and cond, conductivity)

Region	Biomass variable	Water N:P	Mat			CanCov	ln (Cl)	ln (cond)
			P/AFDM	N/AFDM	N:P			
Knobs	ln (ben chl <i>a</i> )	-0.045	-0.184	-0.156	0.100	<b>0.374</b>	<b>0.540</b>	<b>0.419</b>
Knobs	ln (dia rank)	0.039	-0.036	-0.091	-0.097	<b>0.351</b>	0.057	0.090
Knobs	ln (Clad cov)	0.049	-0.047	-0.118	-0.127	0.196	<b>0.422</b>	<b>0.538</b>
Glaciated	ln (ben chl <i>a</i> )	-0.058	0.246	<b>0.412</b>	0.111	-0.23	<b>0.314</b>	<b>0.386</b>
Glaciated	ln (dia rank)	-0.08	-0.070	-0.254	-0.193	-0.228	<b>0.327</b>	<b>0.421</b>
Glaciated	ln (Clad cov)	-0.141	-0.115	0.163	0.244	-0.072	<b>0.583</b>	<b>0.589</b>
Both	ln (ben chl <i>a</i> )	0.028	0.185	<b>0.463</b>	<b>0.273</b>	-0.129	<b>0.260</b>	0.091
Both	ln (dia rank)	0.041	0.180	<b>0.322</b>	0.211	0.114	0.100	-0.036
Both	ln (Clad cov)	-0.020	0.008	0.123	0.154	0.073	<b>0.501</b>	<b>0.493</b>

Correlation coefficients (*r*) in bold indicate statistical significance with  $p < 0.05$ .

Canopy cover was positively correlated ( $p < 0.05$ ) to chl *a* and diatom rank in KY, but was not related to algal biomass in other cases (Table 3). Even *Cladophora* cover was not related significantly to canopy cover. Correlations between chl *a*/AFDM ratios and canopy cover were  $r = 0.444$  in KY and  $r = -0.014$  in MI.

Chloride concentration and conductivity were positively correlated ( $p < 0.05$ ) with measures of algal biomass in both KY and MI. Correlations between these indicators of human disturbance and algal biomass were usually higher than correlations between nutrient concentrations and algal biomass (Table 3).

#### Comparison of biomass–nutrient models between regions

Algal biomass was usually lower in MI than KY, as indicated by the significantly lower constants in MI than KY in TP models (Fig. 5, Table 4). However, responses of benthic algal biomass to nutrients seemed to be due to changes in *Cladophora* versus epilithic diatom biomass, and these were higher in MI than KY. Chl *a* increased with nutrients in both regions at approximately the same rate, approximately  $0.6 \mu\text{g chl } a \text{ cm}^{-2} \text{ per ln } (\mu\text{g l}^{-1})$  of both TP and TN. Diatom thickness rank did not increase significantly with nutrients in either region, however *Cladophora* cover did. TN and TP models indicated that *Cladophora* cover was lower in low nutrient conditions in the MI than KY, but increased faster with nutri-

ent enrichment in MI. The constants for models relating TN and TP with *Cladophora* cover were significantly lower in the MI than KY for both TN and TP models, but slopes for these models were higher in the MI than KY. The slope for the *Cladophora*/TP model was not significantly different than 0.0 in KY ( $p = 0.058$ ).

#### *Cladophora* cover

Due to differences in *Cladophora*/nutrient models between regions, differences in percent cover in different nutrient regimes were examined more closely (Figs. 6 and 7). The probability of observing average *Cladophora* cover  $< 1\%$  in a stream was negatively related (Spearman rank correlations,  $p > 0.05$ ) to nutrient concentrations in MI, but not in KY. However, *Cladophora* accrual was severely constrained in low nutrient streams in both regions. Average *Cladophora* cover was less than 1% in all streams of MI having less than  $300 \mu\text{g TN l}^{-1}$  and  $10 \mu\text{g TP l}^{-1}$ . Thirty and fifty percent of KY streams in that low nutrient category ( $300 \mu\text{g TN l}^{-1}$  and  $10 \mu\text{g TP l}^{-1}$ ) had, respectively, average and maximum *Cladophora* cover  $< 1\%$ . In addition, only 1 of 25 of these low nutrient KY streams had average *Cladophora* cover  $> 10\%$  and only 3 of 25 had maximum cover  $> 10\%$ .

The probability of maximum *Cladophora* being  $> 40\%$  increased significantly (Spearman rank correlations,  $p > 0.05$ ) with both TN and TP conditions in both regions. The probability of average *Cladophora* cover being greater than 10%

Table 4. Linear regression statistics for relationships between six measures of algal biomass and nutrient conditions in streams of KY and MI

Dependent variable	Independent variable	Region	Constant	Constant (SE)	Slope	Slope (SE)	Model <i>p</i>
ln (chl <i>a</i> )	ln (TN)	KY	-2.098	1.035	0.62	0.16	< 0.001
ln (chl <i>a</i> )	ln (TN)	MI	-3.859	1.625	0.639	0.247	0.012
ln (chl <i>a</i> )	ln (TN)	both	-2.389	1.07	0.535	0.164	0.001
ln (chl <i>a</i> )	ln (TP)	KY	<b>0.408</b>	0.422	0.521	0.239	< 0.001
ln (chl <i>a</i> )	ln (TP)	MI	<b>-1.908</b>	0.813	0.72	0.256	0.006
ln (chl <i>a</i> )	ln (TP)	both	-0.167	0.471	0.42	0.153	0.007
ln (diatom rnk)	ln (TN)	KY	0.298	0.214	0.049	0.033	0.137
ln (diatom rnk)	ln (TN)	MI	0.155	0.188	0.041	0.028	0.15
ln (diatom rnk)	ln (TN)	both	0.317	0.172	0.035	0.026	0.18
ln (diatom rnk)	ln (TP)	KY	<b>0.529</b>	0.089	0.03	0.029	0.299
ln (diatom rnk)	ln (TP)	MI	<b>0.245</b>	0.093	0.06	0.03	0.053
ln (diatom rnk)	ln (TP)	both	0.467	0.074	0.027	0.024	0.269
ln (avg Clad cov)	ln (TN)	KY	<b>-1.287</b>	0.953	<b>0.369</b>	0.146	0.014
ln (avg Clad cov)	ln (TN)	MI	<b>-6.717</b>	1.875	<b>1.179</b>	0.611	< 0.001
ln (avg Clad cov)	ln (TN)	Both	-2.583	0.881	0.564	0.136	< 0.001
ln (avg Clad cov)	ln (TP)	KY	<b>0.367</b>	0.4	<b>0.251</b>	0.13	0.058
ln (avg Clad cov)	ln (TP)	MI	<b>-3.667</b>	0.848	<b>1.567</b>	0.273	< 0.001
ln (avg Clad cov)	ln (TP)	Both	-0.317	0.385	0.48	0.125	< 0.001

The relations between biomass and TP differ from those illustrated in Figure 4 due to limiting the TP range to <100  $\mu\text{g l}^{-1}$ . SE, standard error. Bold statistics indicate a statistically significant difference ( $p < 0.05$ ) between KY and MI using either pooled estimates of variance or individual estimates of variance. Bold statistics in italics indicate a statistically significant relationship between KY and MI using pooled estimates of variance only.

increased with TN and TP conditions in MI, but not KY. Between 20 and 50% increases in the likelihood of high *Cladophora* cover (>20% average and >40% maximum cover) were observed in MI when TP exceeded 30  $\mu\text{g TP l}^{-1}$  and 1000  $\mu\text{g TN l}^{-1}$ . Similarly great increases were not observed in KY, but clear evidence of positive relationships between likelihood of high *Cladophora* cover and TP and TN concentrations during peak accrual periods were observed there (Figs. 6 and 7).

## Discussion

### *Algal–nutrient relations*

Variation in benthic algal biomass among streams was related to nutrient concentrations, but some relationships changed with region and between diatoms and the macroalga *Cladophora*. Total benthic algal biomass increased in both MI and KY with increasing concentrations of both soluble

and total nutrient concentrations. However, most of the predictable increase in biomass was probably due to an increase in *Cladophora* cover. Epilithic diatom biomass did not increase significantly with increasing nutrient concentrations in either region.

Algal biomass was similarly related to TN, TP, and soluble nutrients (except  $\text{NH}_4^+$  concentration). Dodds (2003) recently reviewed the rationale for total nutrients being better indicators of nutrient supply than soluble nutrients and clearly relates nutrient demand, uptake, and empirical evidence to this issue. However, Biggs (2000) was able to relate benthic algal biomass to soluble nutrients by using annual means of dissolved nutrient concentrations as an indicator of nutrient supply and accounting for time of accrual after disturbance. Our two-month averages in nutrient concentrations in KY streams may also have provided an indicator of nutrient supply. However, the lower variability in algal–nutrient relations in our study than in other large-scale regional studies (Bourassa & Cattaneo, 1998; Dodds et al., 1998, 2002; Biggs,

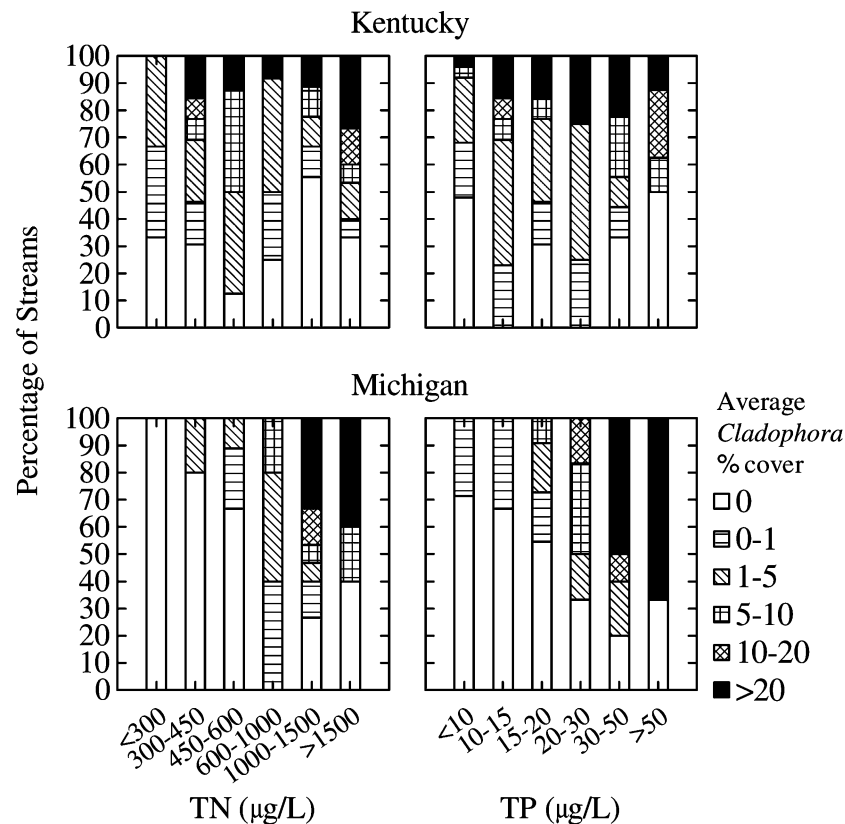


Figure 6. Histograms representing the percentage of streams in different nutrient categories and in different regions with increasing cover of *Cladophora*. Width of the bands in each bar represents the percentage of streams in a nutrient category with specific ranges of average *Cladophora* cover. Codes for those ranges are in the legend.

2000) is probably due to other factors as well, such as distinguishing responses of diatoms and *Cladophora* and controlling for effects of in-stream spatial variation and regions.

Differing responses of diatoms and *Cladophora* may be due to differences in physiological requirements for nutrients or to differences in density-related constraints of nutrient supply through thick diatom and *Cladophora* accumulations. Accrual of even thick growths of diatoms is saturated in experiments around  $30 \mu\text{g P l}^{-1}$  (Bothwell, 1989; Rier & Stevenson, accepted), and the negative effects of mat density on solute mixing, nutrient supply rate, and benthic algal growth rates are great (Stevenson et al., 1991; Stevenson & Glover, 1993). Little is known about effects of specific nutrient concentrations on *Cladophora* growth rates in streams (Dodds & Gudder, 1992). The ability of *Cladophora* to develop thick, deep growths in streams (Dodds, 1991) and thick

epiphyte covers may increase the demand of *Cladophora* for nutrients more than diatom-dominated periphyton.

The lack of response of diatoms to nutrients in either region was unexpected, especially in KY where diatom accrual was sometimes great. Greater variability in diatom biomasses in KY than MI were observed. Many KY streams had less than  $30 \mu\text{g P l}^{-1}$  and  $300 \mu\text{g N l}^{-1}$ , which have been shown to limit accrual of peak biomasses of diatoms in experimental settings (Bothwell, 1989; Rier & Stevenson, accepted). Even though error in nutrient assessments of streams was not sufficient to mask other algal–nutrient relationships, it may have masked diatom–nutrient relationships. Alternatively, high biomasses of algae can accumulate in low nutrient conditions if loss rates are low (e.g., Stevenson et al., 2002).

Differences in benthic diatoms between regions in our study were probably due to grazing by

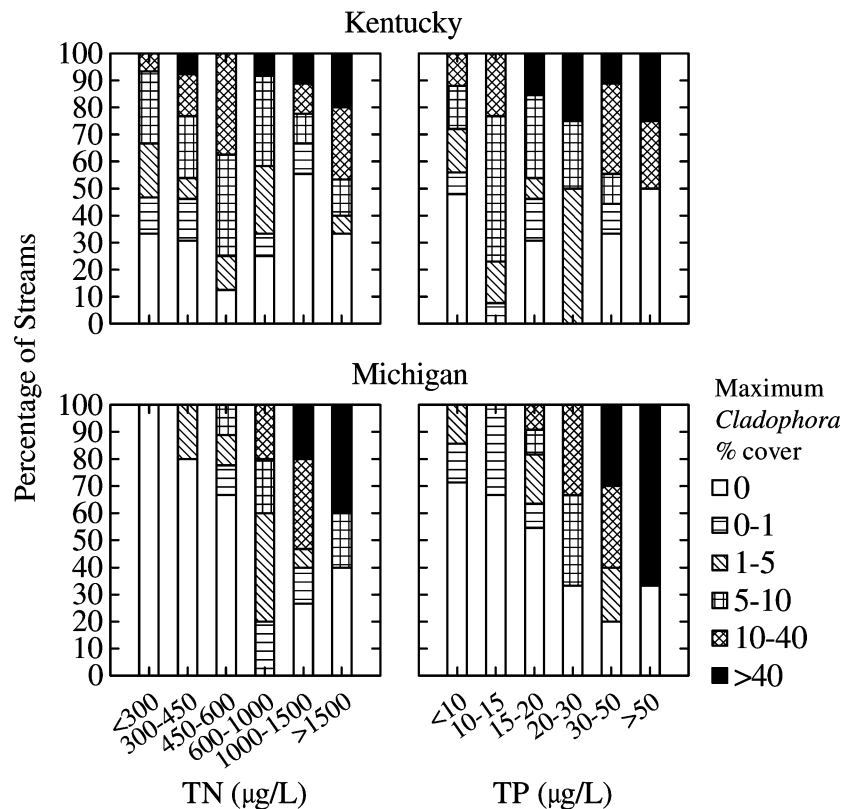


Figure 7. Histograms representing the cumulative frequency of streams in different nutrient categories and in different regions with increasing cover of *Cladophora*. Width of the bands in each bar represents the percentage of streams in a nutrient category with specific ranges of maximum *Cladophora* cover. Codes for those ranges are in the legend.

invertebrates. Benthic invertebrate biomass was much lower in streams of KY than MI. Low invertebrate biomass in the KY has been related to the more intense natural disturbance regime due to flooding and summer drought (Riseng et al., 2004). Invertebrate grazers are commonly associated with regulation of diatom-dominated periphyton (Lamberti & Resh, 1983; Feminella & Hawkins, 1995) and it is well documented in MI streams (Kohler & Wiley, 1997). Although averages of many environmental conditions were statistically different between regions, their ranges overlapped greatly. It's unlikely that the statistically significant differences in water temperature, pH, alkalinity, conductivity, and canopy cover were sufficient to be biologically significant and affect diatom or *Cladophora* biomass. Ranges in nutrient concentrations were very similar in the two regions.

Relationships between algae and nutrient concentrations varied greatly between regions and

were probably related to inaccuracies in measuring nutrient availability, herbivory, and scouring by floods. Correlations between measures of algal biomass and nutrients were higher when observations were constrained to one region or another versus when data for both regions were combined. Although diatom biomass did not increase with nutrients in either region, *Cladophora* cover did increase with nutrients and more rapidly in MI than KY. The soluble fraction of TP was greater in KY than MI, so the greater responses of *Cladophora* to low nutrients in KY than MI may be due to a higher fraction of bioavailable P. Grazers can consume *Cladophora* (Dudley & D'Antonio, 1991), thus lower % cover of *Cladophora* in low nutrient streams of MI than KY (Figs. 5 and 6) may have been due to higher grazing pressure. Higher likelihood of extensive growths in high nutrient streams of the MI than KY may be due to less frequent flood disturbances in MI.

Disturbance regime has been related to algal biomass in streams by Biggs (1995), who suggested that high disturbance regimes constrain algal biomass. This prediction seems to conflict with observations of lowest algal biomass in the low disturbance regimes of our glaciated region and below dams in other studies (Wootton et al., 1996). However, the mechanisms regulating the algal–nutrient relationships in different disturbance regimes and the range of disturbance regime probably account for differences in predictions. The Biggs model was developed in very high disturbance rivers draining high gradient mountain streams, which probably have more frequent and intense disturbances than the Knobs region of KY. Frequent, intense disturbances probably reduce the ability of periphyton to recolonize between storm events by scouring algae off substrata and preventing substantial recolonization. In the very low disturbance regimes of groundwater-fed, hydrologically stable streams in glaciated regions, we find high densities of grazers constraining diatom accrual. Thus, the greatest response of benthic algal biomass to nutrients is most likely at intermediate levels of disturbance, where sufficient time occurs between storm events for algal recolonization but not enough time for regrowth of sufficient invertebrate densities to constrain algal accrual. (Fig. 8)

#### *Nutrient criteria*

The primary purpose of establishing nutrient criteria is to prevent nuisance growths of algae. Nuisance growths may be related to benthic diatoms as well as proliferation of filamentous green algae in streams (Biggs, 2000). Response of *Cladophora* to nutrients should be given special consideration when establishing nutrient criteria to prevent in-stream nuisance algal growths. Filamentous green algae are prevalent in high biomass conditions (Chetelat et al., 1999), affect aesthetics, may cause local DO-depletion, and alter habitats in ways that affect invertebrates and fish (Dudley et al., 1986).

Two important issues for establishment of nutrient criteria are identifying which nutrient limits algal growth and determining the concentration of that nutrient enabling nuisance growths. Phosphorus is usually identified as the nutrient

limiting algal growth in streams, however experiments show that N may also be a factor (Francoeur, 2001). Strength of correlations between biomass and nutrients and nutrient ratios has been used as evidence for likely N or P limitation. Using strength of correlations to indicate limiting nutrients in streams is problematic in streams because high benthic biomass may result in depletion of the most limiting nutrient in the water column. The negative relationship between Si and algal biomass in KY where high diatom biomasses develop demonstrates that nutrient demand is sufficient to deplete water column resources. The high correlation between  $\text{NH}_4^+$  concentration and algal biomass could indicate N limitation;  $\text{NH}_4^+$  concentrations were very low, relative to  $\text{NO}_x$  and TN and  $\text{NH}_4^+$  is assumed to be the preferred form of dissolved N. More likely,  $\text{NH}_4^+$  is a good indicator of nutrient loading and supply by human activities, like CI and conductivity.

Water and mat N:P ratios, P/AFDM, and N/AFDM were poorly related to algal biomass in our study. The positive correlation between N/AFDM and diatom biomass was probably due to correspondence in regional differences in both N/AFDM and algal biomass rather than an indicator of N regulation of algal biomass. Both N/AFDM and diatom biomass were lower in MI than KY, but the low biomass in MI was most likely due to herbivory. Low N:P ratios in stream water were not related to algal biomass, which was probably again caused by the complexity in relationships between nutrient ratios, loading rates, absolute water column concentrations, and uptake rates by benthic algae (Stelzer & Lamberti, 2001; Snyder et al., 2002). Inclusion and variability in detritus, bacteria, and meiofauna may affect use of N:P ratios in periphyton samples to estimate algal N:P ratios in field studies.

Low average N:P ratios in periphyton indicated the possibility of N-limitation of algal accrual. Average N:P ratios in periphyton were 11 and 9 in KY and MI, which is less than Redfield ratio and much less than the 103 and 84 N:P ratios in KY and MI waters. Humphrey & Stevenson (1992) showed molar N:P ratios decreasing from 13.4 to 3.1 during periphyton community development in experimental streams, which was hypothesized to be due to greater cellular retention and within-mat

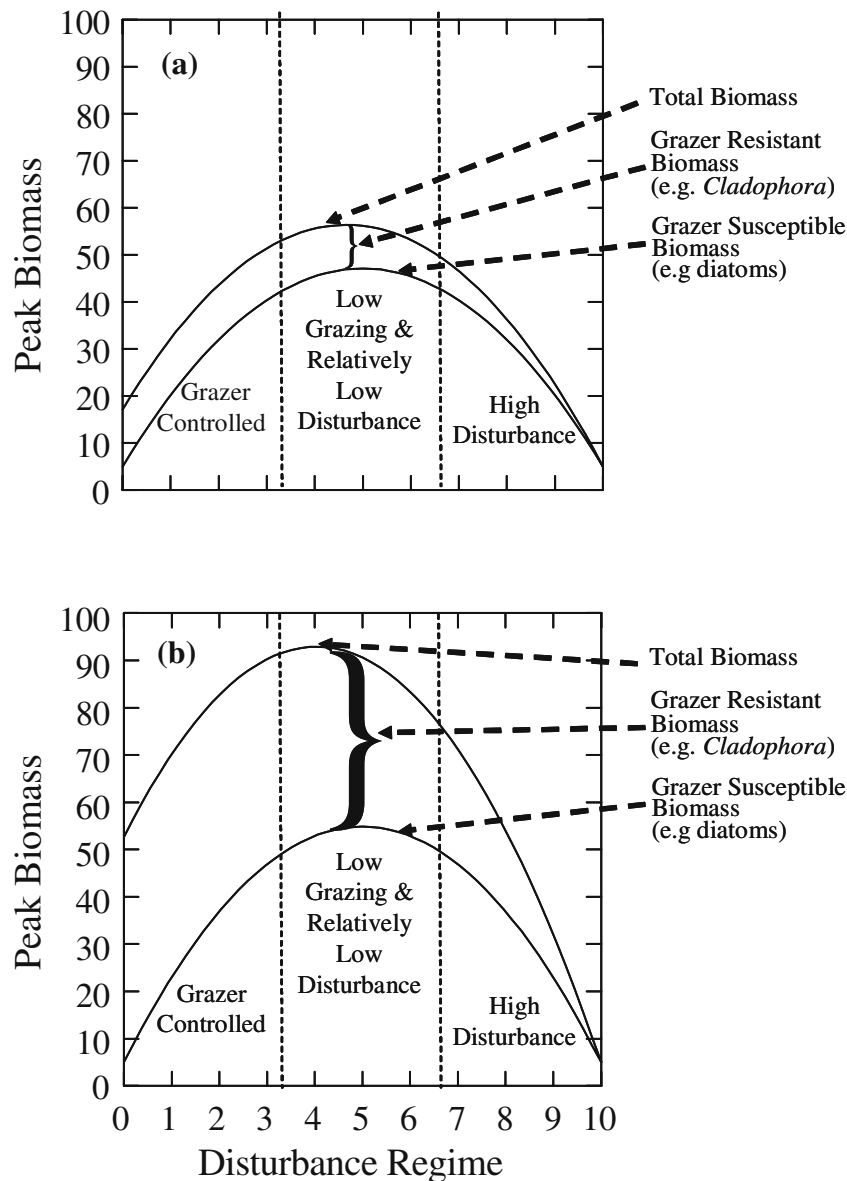


Figure 8. Conceptual model of effects of physical disturbance regime on peak biomass of grazer susceptible algae, grazer resistant algae (such as *Cladophora*), and all benthic algae in relatively low (a) and high (b) nutrient conditions. Grazer density is hypothesized to be high in low disturbance regimes, where grazers regulate accumulation of grazer-susceptible algae (such as diatoms). In high nutrients and low disturbance, grazers cannot regulate density of grazer resistant forms, such as *Cladophora*. Grazer density decreases with disturbance (Riseng et al. 2004) and enables accrual of both grazer-susceptible and resistant forms in habitats with intermediate levels of disturbance. In high disturbance, accrual of both grazer-susceptible and grazer-resistant forms are constrained physical disturbance.

recycling of P than N. Thus, initial algal accrual after disturbance may be regulated by P availability, but sustained accrual to reach high biomass may depend on N availability. In addition, N and P limitation may vary seasonally with seasonal differences in uptake and retention of nutrients by

terrestrial vegetation in temperate zones (Meyer et al., 1988). Criteria for N as well as P may be useful for constraining accumulations of nuisance algal growths.

We did not find thresholds in algal biomass–nutrient relations, which can be predicted

based on models and can be helpful in establishing criteria for protecting valued ecological attributes (Stevenson, 1997a; Muradian, 2001; Stevenson et al., in press). We found some evidence of diatoms and *Cladophora* escaping from grazing pressure in high nutrient concentrations in the glaciated MI region, but little evidence of saturating nutrient concentrations at high N and P levels. The great spatial and temporal variability in measures of algal biomass and nutrient availability may have masked non-linear relationships between biomass and nutrients. In addition, saturation of algal growth by N and P at high concentrations may be complicated by the increasing nutrient demand that likely results as biomass increases and constrains nutrient transport and availability (Stevenson & Glover, 1993; Rier & Stevenson, accepted).

Without threshold responses in algal–nutrient relationships, we need to rely more on the reference approach for criteria development (Hughes & Larsen, 1986; Hughes, 1995). Streams in both MI and KY with the lowest levels of human disturbance in watersheds (reference streams,  $n=6$ ) had  $TP \leq 11 \mu\text{g l}^{-1}$  and  $TN \leq 400 \mu\text{g l}^{-1}$ . Nutrient concentrations in our study streams increased with % agriculture and decreased with % forest in both regions (Riseng et al., 2004; Wang & Stevenson, accepted). Given our observed probability of *Cladophora* cover in low nutrient, reference streams, *Cladophora* accrual would be very low in the most natural streams. Streams with low nutrient conditions in the EMAP study of the Mid-Atlantic region (unpublished data) also had very little occurrence of filamentous algae. In MI, diatom rank was constrained to 0.5 in reference streams, which corresponded to a chl *a* concentration of  $\approx 1.0 \mu\text{g cm}^{-2}$ ; but a wider range of diatom ranks were observed in KY. Figure 5 indicates benthic chl *a* would be about 1 and  $2 \mu\text{g cm}^{-2}$  in reference streams of MI and KY, respectively, which is very similar to the definition of oligotrophic streams by Dodds et al. (1998). However, the TP and TN concentrations in our reference streams are about half of the concentrations recommended by Dodds et al. (1998) as the oligotrophic–mesotrophic boundary.

High algal biomasses, for example average *Cladophora* cover  $>20\%$  or  $>40\%$  maximum cover were rare (less than 10% of streams) in both

regions, if TP was less than  $30 \mu\text{g l}^{-1}$  and TN was less than  $1000 \mu\text{g l}^{-1}$ . The  $30 \mu\text{g TP l}^{-1}$  target was also recognized by Dodds et al. (1998) as a concentration that constrained chl *a* to less than  $15 \mu\text{g chl a cm}^{-2}$  in a *Cladophora* dominated stream. Higher probabilities of more extensive *Cladophora* growths were observed with increasing nutrient levels. Thus,  $30 \mu\text{g TP l}^{-1}$  and  $1000 \mu\text{g TN l}^{-1}$  could be considered as targets to prevent a high probability of nuisance accrual of *Cladophora*.

Thus, different nutrient criteria could be established to support stream-specific management goals. The concept of “tiered aquatic life uses” has been suggested to enable protection of high quality waters as well as goals for restoring impaired ecosystems (S.B. Davies & S.K. Jackson, submitted). To protect naturally low levels of productivity and algal biomass in streams that are hydrogeomorphically similar to our study streams, nutrient concentrations should probably be constrained to  $\leq 10 \mu\text{g TP l}^{-1}$  and  $400 \mu\text{g TN l}^{-1}$ . To prevent nuisance growths of *Cladophora*, higher criteria in the range of  $30 \mu\text{g TP l}^{-1}$  and  $1000 \mu\text{g TN l}^{-1}$  may be satisfactory. More research is necessary to refine these criteria, but these concentrations should provide a starting point for adaptive management of nutrients in streams.

In conclusion, relationships between algal biomass and nutrient concentrations in streams varied with the type of algae and the region. Epilithic diatom biomass did not respond as much to nutrient enrichment as *Cladophora* cover. Some evidence suggested N as well as P limitation of high biomass accrual. Different P and N criteria can be identified to support high quality conditions in selected streams and to restore minimum acceptable levels of biological condition. Further refinement of algal–nutrient relationships is warranted to relate algal problems to N as well as P and to other valued ecological attributes, such as taste and odor in drinking water, low DO, and impaired habitat for invertebrates and fish.

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