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Ecological responses to nutrients in streams and rivers of the Colorado mountains and foothills

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SUMMARY

1. Abundance and composition of periphyton and benthic macroinvertebrates were treated as potential nutrient response variables for 74 streams in montane Colorado. The streams ranged from unenriched to mildly enriched with nutrients (N, P).
2. The study showed no meaningful relationship between periphyton biomass accumulation and concentrations of total or dissolved forms of nitrogen or phosphorus. Nutrient concentrations were also unrelated to periphyton and macroinvertebrate richness, diversity and community composition. Macroinvertebrate communities did, however, show a strong positive relationship to periphyton abundance.
3. A positive response of periphyton biomass to increasing nutrient concentrations has been well documented over large ranges of nutrient concentrations. Our study suggests that the nutrient response is suppressed by other controlling factors on the lower limb of the nutrient response curve (i.e. at low nutrient concentrations); a quantitatively significant response occurs only in excess of a threshold beyond which nutrients become dominant over other controlling factors. This interpretation of the results is consistent with published meta-analyses showing lack of nutrient response for a high proportion of experimentally enriched periphyton communities, and division of responses between N and P for communities that do show growth in response to enrichment.
4. Grazing probably is not the key controlling variable for periphyton in Colorado mountain streams, given that the highest chlorophyll concentrations are associated with the highest abundances of macroinvertebrates. Modelling indicates that the initial amount of periphyton biomass at the start of the growing season, in conjunction with elevation-related length of the growing season and water temperature, explains most of the variation in periphyton accumulation among these streams, but there is a yet unexplained suppression of periphyton growth rates across all elevations.

Keywords: attached algae, eutrophication, herbivory/grazing, invertebrates, nuisance algae

Introduction

Substantial nutrient pollution enhances periphyton biomass accumulation in streams with a stable substratum and adequate substratum irradiance (Dodds, 2006), but the controlling role of nutrients in unenriched or mildly enriched streams is not yet well resolved. A periphyton nutrient response can be represented by a curvilinear model that predicts a

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very strong response to nutrients at low nutrient concentrations followed by decreasing responsiveness at higher concentrations, but without any indication of an asymptote even up to very high concentrations (O'Brien *et al.*, 2007). While no definitive mechanistic explanation is yet available for this pattern, possibilities include replacement of taxa that develop only modest biomass per unit area at low nutrient concentrations by other taxa that produce more biomass accumulation but require high nutrient concentrations. In addition, it appears that saturation concentrations of nutrients for periphyton in streams are high when compared to phytoplankton in lakes (Dodds *et al.*, 2002; Mulholland, Steinman & Elwood, 1990; O'Brien *et al.*, 2007). The combination of high saturation concentrations and potential changes in species composition that would support higher biomass may explain the incremental extension of biomass accumulation to very high nutrient concentrations without an asymptote.

Nutrient concentrations for the lower portion of the nutrient response spectrum correspond to oligotrophic [<25 μg L^{-1} total P (TP)] and mesotrophic (<75 μg L^{-1} TP) ranges (Dodds, Jones & Welch, 1998). As shown primarily by use of artificial substrata, the periphyton of streams with low nutrient concentrations show widely varied responses to nutrients. For example, meta-analyses (Dodds & Welch, 2000; Tank & Dodds, 2003; Francoeur, 2001) have shown that about half of nutrient manipulation experiments indicate no response to the addition of phosphorus or nitrogen together or separately. Responses to nutrients, when they occur, are nearly equally divided between separate N or P limitation and colimitation by N and P. Responses typically are stronger for the addition of the two nutrients together than for either nutrient separately (Francoeur, 2001). These response patterns are also typical of lakes (Elser, Marzolf & Goldman, 1990; Lewis & Wurtsbaugh, 2008), except that the phytoplankton of lakes show a stronger growth response at low nutrient concentrations.

The highly variable response of periphyton communities to experimental nutrient enrichment in streams with low nutrient concentrations raises questions about the potential responsiveness of such streams to natural variability in nutrients or to modest degrees of anthropogenic enrichment. For example, nitrogen enrichment by anthropogenic atmospheric nitrogen (nitrate, ammonium) and the liberation of phosphorus and nitrogen through land-use practices could be expected to raise nutrient concentrations by amounts that do not cause streams to qualify as being substantially polluted but could change the mean nutrient concentrations by 25–100%, even without exceeding the natural range of regional variability.

The purpose of this study is to test the hypothesis that substantial amounts of variance in abundance and community characteristics of benthic algae and invertebrates can be explained by concentrations of nitrogen and phosphorus in streams over a large number of montane sites in Colorado. The study focuses on characteristics of benthic communities towards the end of the growing season, when biomass accumulations are near their annual maximum. Because the study does not include sites that are substantially polluted with nutrients, the results and interpretation are applicable to nutrient concentrations that reflect natural variability among sites as well as mild degrees of anthropogenic enrichment.

Methods

Sample collection

The study included 74 sites ranging in elevation from 1500 to 3000 m amsl (Fig. 1). All sites had predominantly natural vegetation and approximately half had no anthropogenic nutrient sources within the catchment, whereas the other half had small nutrient sources associated with low-density housing (septic systems) or livestock grazing. Sites had substratum ranging from gravel to small boulders and some exposed bedrock. All streams were shallow at base flow (<1 m mean depth).

In streams that show repeated removal of periphyton biomass by spates, frequent measurements may be the only practical means of interpreting variability caused by disturbance of the substratum (Biggs, 2000; Cronin *et al.*, 2007). In montane streams of Colorado, however, stripping of periphyton biomass from substrata occurs during the spring runoff. Following runoff, biomass typically builds to an autumn maximum (Fig. 2). Therefore, present comparisons of periphyton accumulation or community composition in streams at various locations are based on samples taken at or near the autumn maximum. Because the growing season ends earliest at high elevation, the sites were sampled in descending order by elevation

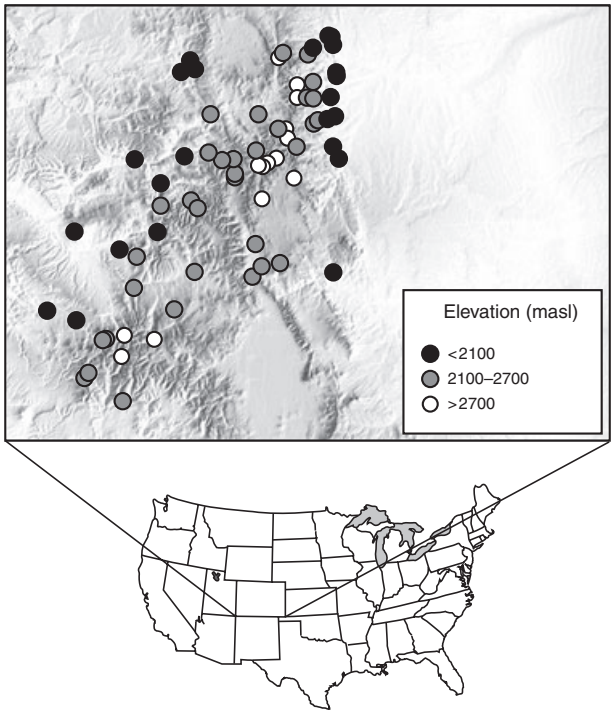

Fig. 1 Map of sampling locations in Colorado segregated by elevation: low (<2100 m), intermediate (2100–2700 m) and high (>2700 m).

category (median dates: alpine, >2700 m, late August; montane, 2100–2700 m, late September; foothills, <2100 m, late October). Data were included for four sites that were sampled by identical methods in 1994–96 (McCutchan & Lewis, 2001; J. McCutchan, unpubl. data).

At each stream or river sampling site, a characteristic reach was identified (25–50 m) within which periphyton and macroinvertebrate samples were collected along four or five evenly spaced transects. Nutrient samples were collected at the same sites on the same dates (analysis as shown in Table 1). Canopy cover (%) at each sampling location was estimated from hemispherical photographs with image-analysis software designed for analysis of canopy cover (five images per site, averaged). The software also estimated photosynthetically available radiation from the measured distribution of shadows, solar path for the days of interest (1 month prior to sampling), elevation and an assumed typical degree of cloud cover (Frazer, Canham & Lertzman, 1999).

Within each stream reach, periphyton were collected from a single stone at three locations (quarter points) along each transect. A bottle cap (25 mm diameter) was placed on the middle of the upper surface of the stone, the area around the cap was scrubbed thoroughly with a nylon-bristle brush, and this area was rinsed with stream water. Then, the cap was removed, the area under the cap was scrubbed with a different brush, and periphyton from beneath

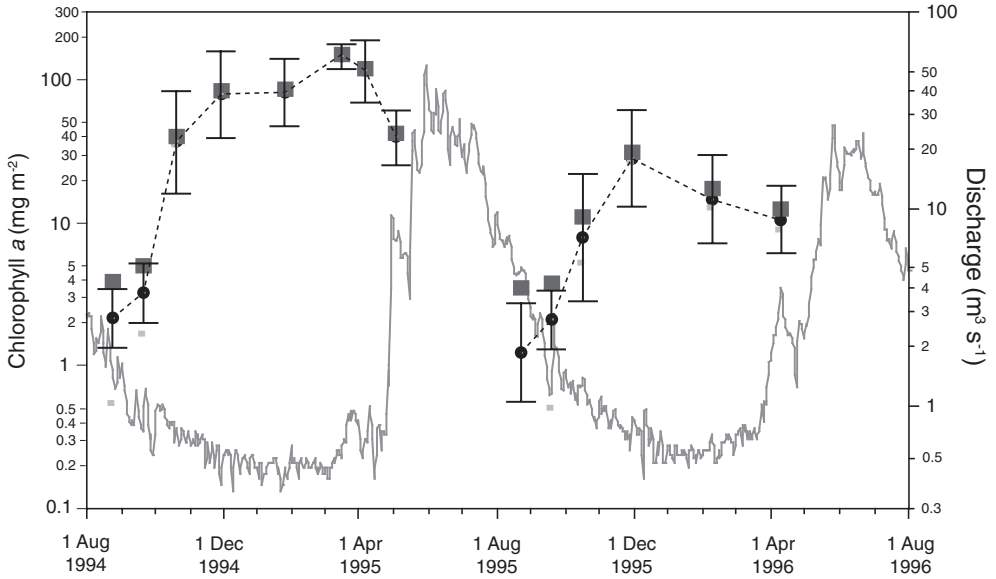

Fig. 2 Illustration typical of the periphyton accumulation pattern in a Colorado montane stream (St. Vrain Creek: J. McCutchan, unpubl. data).

Table 1 Methods used in analysing samples collected in 2004

Analysis	Method	References	Detection limit, $\mu g\ L^{-1}$
SRP	Ascorbic acid-molybdate method	Murphy & Riley, 1962	0.4
TDP	Oxidation followed by ascorbic acid-molybdate method	Lagler & Hendrix, 1982; Valderrama, 1981; Murphy & Riley, 1962	0.8
TPP	Oxidation followed by ascorbic acid-molybdate method	Lagler & Hendrix, 1982; Valderrama, 1981; Murphy & Riley, 1962	0.8
TP	Sum of TDP and TPP		2
Ammonia-N	Modified Solorzano method	Grashoff, 1976	3
Nitrate-N	Ion chromatography		2
TDN	Oxidation with potassium persulfate followed by ion chromatography	Valderrama, 1981; Davi *et al.*, 1993	4
TN	Oxidation with potassium persulfate followed by ion chromatography	Valderrama, 1981; Davi *et al.*, 1993	4
Chl *a*	Spectrophotometric method, ethanol extraction	Marker *et al.*, 1980; Nusch, 1980	1

SRP, soluble reactive P; TDP, total dissolved P; TN, total nitrogen; TP, total P; TPP, total particulate P.

the cap were rinsed into a sample container. Samples from each transect were pooled and diluted with stream water to a measured volume (150–200 mL). Prior to filtration (within 12 h of collection), samples were stored on ice in the dark; 100–200 mL of the pooled sample was passed through a Whatman glass-fibre (GF/C) filter for chlorophyll analysis (Table 1). Filters for chlorophyll analysis were stored in the dark at −20 °C prior to analysis.

Identification and counting of taxa

Periphyton taxa were identified and counted with an inverted microscope. Samples were diluted by measured amounts as needed for counting. Large taxa (>200 μm) were counted over the entire bottom of two chambers at 125×. Periphyton >10–20 μm were counted within fields at 125× magnification, and smaller algae were counted within fields at 500× and 1250×. At least 30 fields were counted at the higher magnifications, and the fields were distributed evenly over two chambers. At least 400 biomass units (colonies, filaments or single cells) were counted per sample, except when fewer than 400 biomass units were present. Algae were identified to the lowest possible taxonomic unit during the count. Diatoms were identified to species after being cleared and mounted; diatom counts were based on chambers rather than on slides.

One sample of benthic macroinvertebrates was collected at each of the four or five transects on each reach, and the samples within each reach were pooled. Samples were collected with one of two modified Surber samplers (250-μm mesh; 0.10 or 0.0625 m^2). The larger sampler was used in deep water (>30 cm), while both samplers were used in shallow water. Invertebrates were subsampled by the random fixed-count method (Plafkin *et al.*, 1989; Canton, 1991; Barbour *et al.*, 1999). Samples were passed through a 355-μm sieve, and remaining large debris was rinsed and removed. Samples were transferred to a Canton subsampling tray with 24 numbered grids (Canton, 1991). For each sample, grids were selected randomly and all organisms were removed under magnification from the grid first selected. Organisms were removed from additional grids until 500 organisms were removed; organisms beyond 500 in the last grid also were removed. Invertebrates were identified to the lowest possible taxonomic level.

Statistical treatment of data

In a stepwise multiple regression analysis for which elevation and date of sampling were treated as independent variables and benthic chlorophyll *a* was treated as a dependent variable, date of sampling entered the regression first. The addition of elevation

as a second independent variable raised the amount of variance accounted for from 38 to 43%; the overall adjusted R^2 for the relationship was 42%. The regression equation was used in correcting benthic chlorophyll *a* to a common date and elevation (median date, 1 October, 2004; median elevation, 2400 masl) for all samples. Similar corrections were made for all other variables showing a significant relationship with date, elevation or both. This procedure would cause underestimates of the influence of nutrients if nutrients were correlated with elevation, but such correlations are either nil or very small in this data set (see Results).

Numerical simulations

A simulation was used to test the consistency of experimental data from published meta-analyses (Francoeur, 2001) with the data obtained in this study. Following the results of the meta-analyses, it was assumed for a simulation involving low nutrient concentrations that 50% of periphyton communities have no nutrient response, 12% have a phosphorus response, 12% have a nitrogen response and 25% respond only to nitrogen plus phosphorus. Variance of peak biomass accumulation was assigned from our study (coefficient of variation equal to 63% for corrected chlorophyll). Variance in response to N or P was assumed to be 50% accounted for by stimulation of growth and 50% by other, undefined factors. A Monte Carlo procedure then was used to create a hypothetical benthic chlorophyll data set for 250 sites.

A second simulation was used to estimate the effect of elevation on periphyton biomass through its relationship to length of growing season and temperature. A large data set (J. McCutchan, multiple years, unpubl. data) containing daily mean temperature for stream sites across the full range of elevations in the Colorado mountains and foothills was used to quantify the variation of water temperature with day of year and elevation. Boundaries on the length of the growing season were set for simulation purposes by spring runoff (the end of which is inversely related to elevation), whose effect is assumed to have subsided to an extent sufficient to allow periphyton growth when peak runoff has declined by 50%, and the date in autumn when mean daily water temperate reaches 4 °C. Net growth rates for periphyton were assumed to be dependent on temperature with a Q_{10} of 2 (Falkowski & Raven, 2007). The average doubling rate at 20 °C was set empirically so that the predicted final biomass matched the observed biomasses for this study. Initial biomass was set to 0.1 mg chl a m^{-2}, corresponding to erosive removal of most overwintering biomass by spring runoff.

Results

Nutrient concentrations

Although the ranges of N and P concentrations reached or exceeded two orders of magnitude across sites, they did not include concentrations typical of highly enriched waters (P > 500 μg L^{-1}; N > 2000 μg L^{-1}, Table 2, Fig. 3). The fractions used here in statistical analysis are soluble reactive P (SRP), total dissolved P (TDP), TP, nitrate and dissolved inorganic N (DIN); no significant relationships were found for dissolved organic nitrogen or total nitrogen (TN). Nitrate and DIN are almost identical because ammonia was consistently scarce.

Canopy cover, date of sampling and elevation

Transmittance of light through the canopy, expressed as per cent, did not vary significantly with day of the year, but irradiance reaching the stream surface (*I*, mol m^{-2} day^{-1}) did decrease substantially with day of the year (D): $I = 77.4–0.0184$ (D), $r^2 = 0.40$, $P < 0.0001$. Sites varied substantially in canopy cover (*c.* 30–95% transmittance), but variation in incident irradiance at the stream surface over the course of the study was related to changes in solar angle and day length and not to systematic variation in canopy cover with elevation. Elevation has a direct effect on irradiance for any given date, but it is far smaller than the effect

Table 2 Summary of nutrient concentrations for the 74 sites

	Mean, μg L^{-1}	Median, μg L^{-1}	Range, μg L^{-1}
Soluble reactive P	10.4	2.7	0.4–195
Total dissolved P	13.9	5.3	1.0–215
Total particulate P	12.2	4.2	0.9–353
Total P	26.5	10.7	3.1–413
Nitrate-N	114	5.3	0–1152
Ammonia-N	10.6	7.3	3.1–105
Total dissolved N	280	229	103–1554
Total N	376	325	163–1615

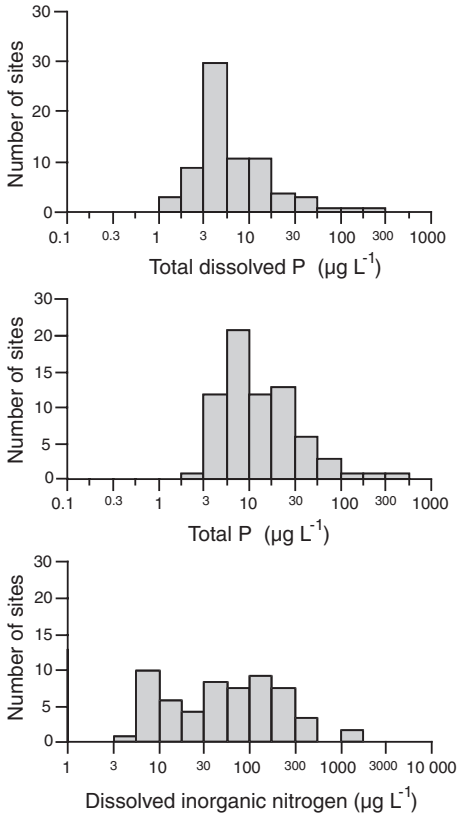

Fig. 3 Frequency distributions of selected nutrient fractions for streams sampled in the study (74 sites). Dissolved inorganic N is mostly nitrate.

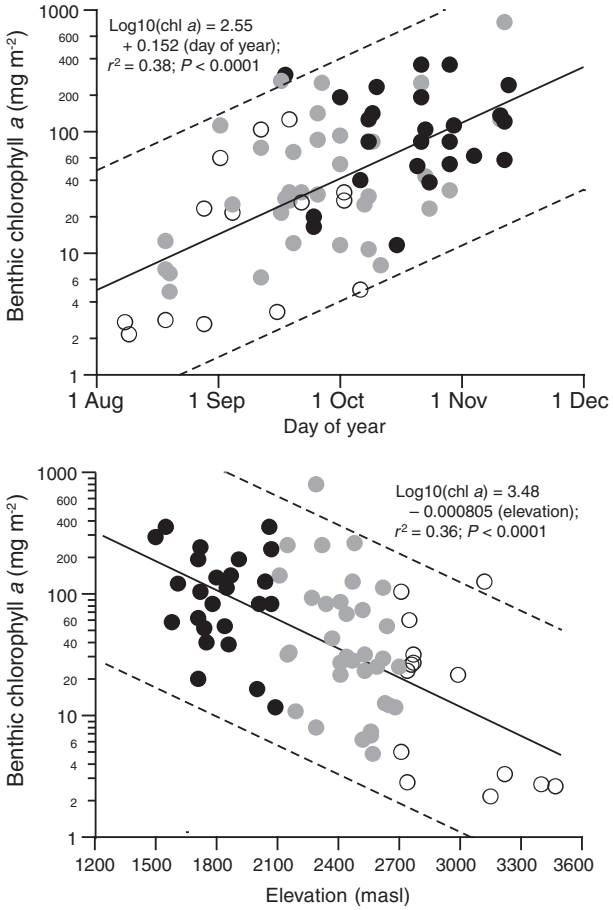

Fig. 4 Benthic chlorophyll *a* in relation to date of sampling and elevation.

of sampling date in this study. Sampling date explains the trend in irradiance, and canopy cover explains much of the scatter around the trend.

In stepwise multiple regression, concentrations of TDP showed a significant but weak relationship with elevation ($r^2 = 0.16$) but date accounted for no additional variance. Neither TP, TN nor any other fraction of P or N showed any significant relationship with elevation or sampling date.

Periphyton abundance

Benthic chlorophyll *a* increased significantly with date of sampling and decreased with elevation (Fig. 4) but showed no relationship with total P, SRP or TDP (Table 3). Chlorophyll showed a statistically significant relationship with DIN, but only a small amount of variance is accounted for by the regression (15%; Fig. 5). The slope of the DIN response is low; the difference between a low DIN location (1 μg L^{-1}) and high DIN location (1000 μg L^{-1}) is about 5 mg m^{-2} benthic chlorophyll *a*, whereas the scatter around any given location of the line at 95% probability is approximately twice this amount.

Periphyton composition

Statistical relationships (Table 3) between nutrients and periphyton numerical abundance, diversity (Shannon–Weaver Index) and richness (number of taxa) were tested by a procedure identical to the one used for chlorophyll *a*. Numerical abundance and species richness showed a significant but very weak relationship to TDP. No other relationships involving nutrients were statistically significant. The ratios of the three dominant division-level taxa (Table 4), when plotted as a function of nutrient concentration, showed no clear patterns.

Table 3 Statistical dependence (r^2) of periphyton and benthic invertebrate community metrics on nutrient concentrations and chlorophyll. Dash indicates not significant at $P = 0.05$

	Concentrations				
Dependent variable	TP	TDP	SRP	DIN	Chlorophyll
Chlorophyll*	–	–	–	0.15	1.00
Algal abundance*	–	0.08	–	–	0.38
Algal diversity	–	–	–	–	0.14
Algal richness*	–	0.08	–	–	0.09
Invertebrate abundance*	–	–	–	–	0.36
Invertebrate richness*	–	–	–	–	0.07
Invertebrate diversity*	–	–	–	–	0.07

DIN, dissolved inorganic N; SRP, soluble reactive P; TDP, total dissolved P; TP, total P.
*Corrected to a common date (October 1) and elevation (2400 masl); see text.

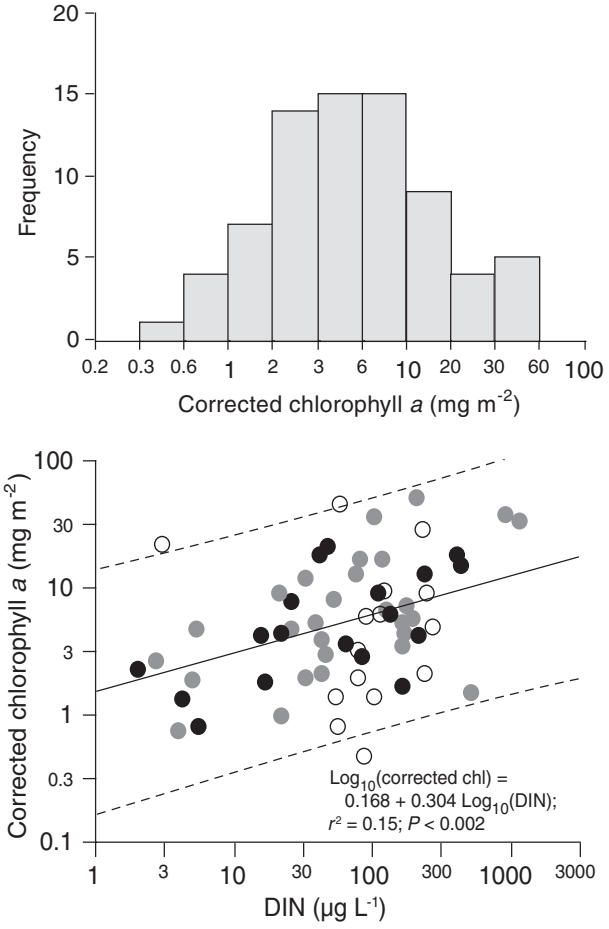

Fig. 5 Frequency distribution of chlorophyll *a* corrected to a common date and elevation (above) and relationship of corrected chlorophyll *a* to dissolved inorganic N (below). Corrected chlorophyll is not related to any other nutrient fractions.

Benthic macroinvertebrates

The field sites supported a diverse benthic macroinvertebrate fauna. The Insecta were dominant numerically with mayflies, caddisflies and dipterans especially important (Table 5). The corrected density of benthic invertebrates was negatively associated with elevation (Fig. 6); there was no added variance accounted for by date when elevation was accounted for. The relationship of corrected chlorophyll to corrected invertebrate density is significant; a change in chlorophyll of three orders of magnitude corresponds to a change in mean expected density of invertebrates of about two orders of magnitude (Fig. 7).

Most herbivorous taxa of benthic macroinvertebrates showed an increase in abundance with increasing chlorophyll, whereas taxa that showed a decrease or no increase were entirely or predominantly predaceous. Thus, the difference in chlorophyll response between herbivorous and predaceous taxa is consistent with a positive relationship between invertebrate density and algal mass (chlorophyll) as a food supply.

Because nutrients may influence the quality of food for invertebrates either by controlling species composition of benthic algae or by the physiological condition of algae, relationships between nutrients and benthic macroinvertebrates also were tested. Abundance of benthic macroinvertebrates showed no indication of any significant statistical relationship with nutrients (Table 3). Macroinvertebrate species richness, expressed as number of families per 500 individuals, and species diversity, quantified using the Shannon–Weaver Index H′, showed significant but very weak relationships with chlorophyll and no relationships with nutrients (Table 3).

Simulations

Simulation 1, which tests the relationship between chlorophyll *a* and the concentration of total phosphorus according to nutrient responses that reflect published

Table 4 Numbers of taxa identified in samples from the 74 sampling locations

	Genera		Taxa		Cell density, cells m^{-2}	
Division	Total	Mean	Total	Mean	Mean	Range of density, cells cm^{-2}
Bacillariophyta	42	13	219	26	2 979 823	2595–23 987 346
Chlorophyta	20	2	34	2	157 001	0–3 891 148
Cyanophyta	23	4	33	5	18 219 982	32 432–80 068 880
Rhodophyta	4	0	4	0	2158	0–73 688
All divisions	88	20	290	34	21 358 964	35 027–108 021 062

Table 5 Numbers of taxa identified in samples from 69 sampling locations

Class	Order	Number of families	Median	Number of taxa	Median	Mean density, m^{-2}	Range of density, m^{-2}
Annelida	Hirudinea	2	0	3	0	3.3	0–154
	Oligochaeta	6	2	12	2	2623	0–78 797
Arachnida	Hydracarina	6	3	8	3	384	0–3072
Crustacea	Amphipoda	3	0	3	0	24	0–768
	Decapoda	1	0	1	0	0.05	0–3
	Isopoda	1	0	1	0	43	0–1459
Elliplura	Collembola	1	0	1	0	0.06	0–4
Hydrozoa	Hydroida	1	0	1	0	4.4	0–307
Insecta	Coleoptera	3	1	9	2	1162	0–10 445
	Diptera	13	5	97	17	7086	310–58 534
	Ephemeroptera	8	4	30	5	3730	0–36 096
	Heteroptera	1	0	1	0	0.87	0–58
	Lepidoptera	1	0	1	0	17	0–307
	Megaloptera	1	0	1	0	0.05	0–3
	Odonata	2	0	2	0	0.77	0–32
	Plecoptera	8	3	20	4	272	0–2114
	Trichoptera	13	4	31	6	2942	6–16 320
Mollusca	Bivalvia	1	0	1	0	42	0–998
	Gastropoda	2	0	5	0	430	0–27 840
Nematoda	Nematoda	1	0	1	0	34	0–600
Platyhelminthes	Turbellaria	2	0	2	0	170	0–5568
Total		80	24	230	42	–	–

meta-analyses and calibration with data from this study, showed a statistically significant but very weak relationship between total P and chlorophyll *a* (Fig. 8). The second simulation, which tests the effects of growing season length and temperature, both calibrated with empirical information from the Colorado mountains, on chlorophyll *a*, showed that elevational trends in these two variables explain large amounts of variation in potential for the accumulation of periphyton biomass over the growing season (Fig. 9).

Discussion

Our study shows no relationship, or only a very weak relationship, between phosphorus or nitrogen and benthic algal density measured as chlorophyll *a*, which seems counterintuitive but is consistent with the literature. The sites for this study fall within the lower limb of the nutrient response curve postulated by O'Brien *et al*. (2007, efficiency loss model) or any similar curve. Curvilinear models portray a steep and nearly linear biomass response to increasing nutrient concentrations, but data from our study show this lower nutrient range to be a zone within which nutrients are very much secondary to other factors in controlling periphyton biomass. Thus, the ascending limb of the response curve is a cloud of points with high variance, whereas the rest of the curve (not quantified here) may be better defined as the product of a state change that occurs when a threshold of nutrient concentration (i.e. change in degree of response to nutrient concentrations) is reached at

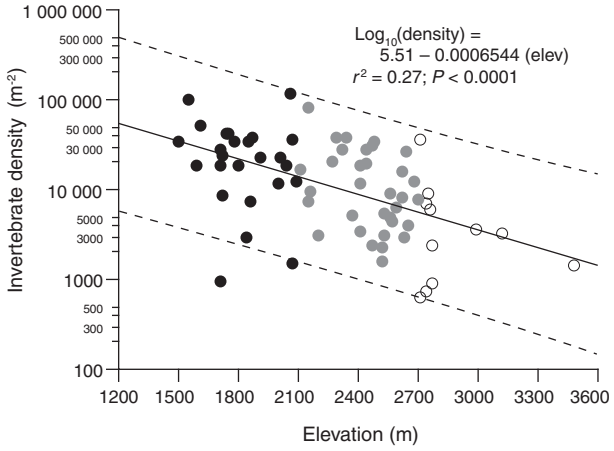

Fig. 6 Relationship between abundance of benthic macroinvertebrates and elevation for samples at 69 sites in 2004 (invertebrates were not sampled at a few of the 74 sites).

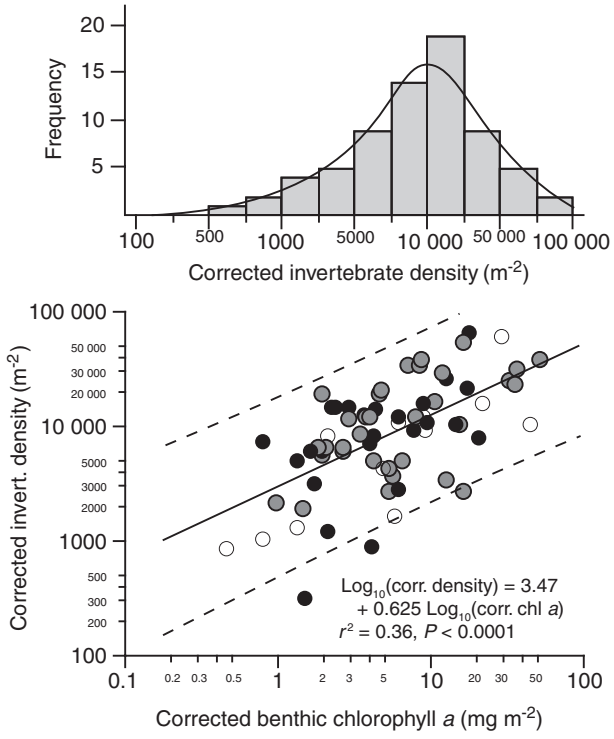

Fig. 7 Frequency distribution of invertebrate density and chlorophyll *a* in relation to invertebrate density.

which growth response to nutrients is high enough to override the factors that control benthic algal abundance at lower nutrient concentrations. In fact, Dodds *et al.* (2002) have given evidence of thresholds (P > 30 μg L^{-1}, N > 40 μg L^{-1}) above which there appears to be a breakpoint to higher chlorophyll. In this study, the striking ineffectuality of nutrient control at low concentrations is underscored by lack of any relationship accounting for more than 10% of variance between algal abundance or community composition and nutrient concentration, even though some regional studies indicate control of periphyton biomass and composition by nutrient concentrations at higher concentrations (e.g. Kovács, Kahlert & Padisák, 2006).

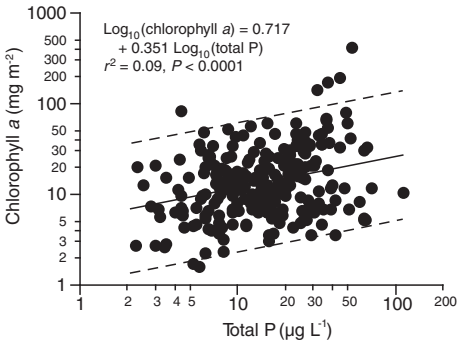

Fig. 8 Simulation results consistent with published meta-analyses, calibrated with variance from our study.

The results of recent meta-analyses for nutrient response based mainly on experiments (Francoeur, 2001) suggest weak or negligible statistical linkages between nutrients and chlorophyll, especially at low nutrient concentrations. Our simulation based on meta-analyses yielded a statistically significant but weak (r^2 = 0.09) relationship between chlorophyll concentration and phosphorus (Fig. 8). This simulation makes predictions that are consistent with the empirical results of our study. The second simulation (Fig. 9) shows that temperature and length of growing season explain a large difference across elevations in the expected (simulated) terminal biomass at the end of the growing season: 10 times initial biomass at the highest elevations versus 1000 times initial biomass at the lowest elevations.

The net growth rates of periphyton across all elevations in this study are much lower than the physiological potential for algal growth at the relevant temperatures, which are as high as one doubling per day (Stevenson, Bothwell & Lowe, 1996), even though there is no evidence of growth rate control through nutrients. Control of net growth rates by grazing could explain growth suppression, but density of grazers is associated positively rather than negatively with chlorophyll. Overall, this study shows that the master variables in unpolluted or minimally polluted

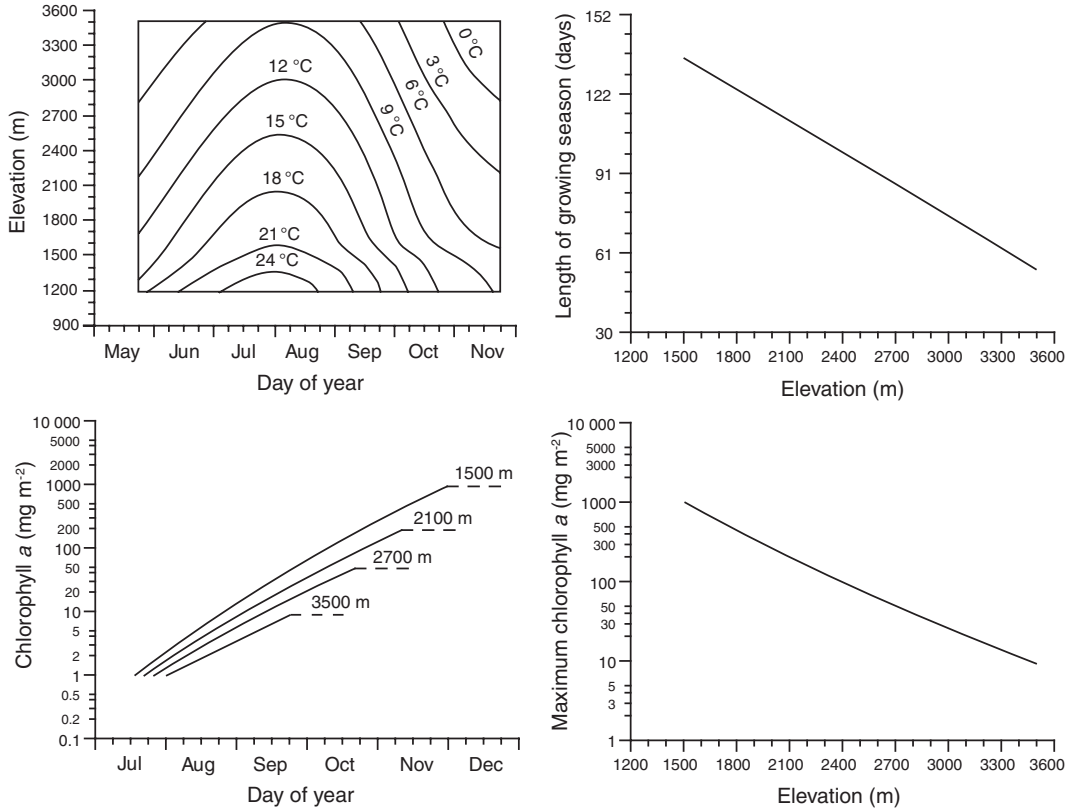

Fig. 9 Basis for simulation of the effects of elevation on water temperature and day length (top panels) and simulation results based on empirical data from our study (bottom panels).

Colorado montane streams are temperature and length of the growth season (both controlled by elevation) rather than nutrients and that there is a yet unexplained suppression of the mean growth rate across all elevations superimposed on the full range of temperatures and growing season lengths.

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