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Published in:
Freshwater Science

DOI:
[10.1899/11-163.1](https://doi.org/10.1899/11-163.1)

Published: 01/12/2012

Document Version
Publisher's PDF, also known as Version of record

[Link to publication](#)

Citation for published version (APA):

Townsend, S., Garcia, E., & Douglas, M. (2012). The response of benthic algal biomass to nutrient addition over a range of current speeds in an oligotrophic river. *Freshwater Science*, 31(4), 1233-1243.
<https://doi.org/10.1899/11-163.1>

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The response of benthic algal biomass to nutrient addition over a range of current speeds in an oligotrophic river

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Abstract. Current speed could affect algal responses to river eutrophication and flow regulation via its influence on nutrient availability to benthic algae. In laboratory experiments, the thickness of the diffusive boundary layer decreases with increasing current speed, enhancing rates of nutrient mass transfer by molecular diffusion across the layer to the algal cell wall. In rivers and streams, this phenomenon may be masked by grazing and physical losses. We used a 10-wk field experiment to test the hypothesis that the response of benthic algae to nutrient addition was a function of current speed. We deployed clay pavers at the beginning of a 3-mo period of baseflow in the Daly River (tropical Australia) at 5 sites (current speeds <2–98 cm/s). After a 5-wk colonization period, we added nutrients to treatment sites via slow-release fertilizer pellets. After 5 wk, we measured chlorophyll *a*, algal composition, and macroinvertebrate grazer abundance and composition, nutrient concentrations, and current speed. We measured algal biomass response (BRR) as the ratio of biomass in the nutrient-addition treatment to biomass in the control treatment. BRR increased linearly with current speed between 27 to 98 cm/s and accounted for 99% of biomass variation. At <2 cm/s BRR did not conform to this relationship. The dominance of filamentous chlorophytes, oligotrophic conditions, and weak grazer effects probably contributed to the strong relationship between current speed and BRR. The effect of nutrient pollution on riverine benthic algal biomass could be greatest where current speeds are highest, and flow regulation that reduces current speed could reduce nutrient availability. Algal composition and physiognomy, grazing, and physical losses could reduce this effect. Current-mediated nutrient availability and patchiness of nutrient-driven bottom-up control of benthic algal biomass may influence algal responses to eutrophication and flow regulation.

Key words: eutrophication, flow regulation, benthic algae, bottom-up control, diffusive boundary layer, velocity, filamentous algae, patchiness.

In riverine systems, current speed varies spatially and temporally over a range of scales, and influences benthic algal biomass via several mechanisms (Hart and Finelli 1999). A positive effect of higher current speed on benthic algal nutrient uptake and growth has been hypothesized for several decades (Whitford and Schumacher 1961, McIntire 1966) and is becoming better understood (Riber and Wetzel 1987, Sanford and Crawford 2000, Larned et al. 2004). As current speed increases, the thickness of the viscous sublayer (diffusive boundary layer [DBL]) that surrounds periphyton and protruding algal cells decreases. This decrease enhances rates of nutrient mass transfer by molecular diffusion across the layer to the algal cell

wall. Nutrient concentrations may be uniform along a reach with spatially variable flow (e.g., pool-riffle-run sequence), but nutrient availability to benthic algae will vary in accordance with current speed. By inference, nutrient-limited algal growth could be increased by increasing current speed.

Current-mediated nutrient uptake by benthic algae could have significant implications for the manifestation of 2 major anthropogenic influences on riverine ecological condition: eutrophication (see Smith 2003) and flow regulation (see Poff et al. 1997) that modifies natural flows and, indirectly, current speeds. Whether current-mediated effects on algae are observable in the field has not been examined. Field experiments done to test the interactive effects of nutrient addition and other factors have most commonly been designed to address light and herbivory (Rosemond et al. 1993, Rosemond 1994, Lange et al. 2011). Flow may be

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included as an environmental variable or cofactor (Matthaei et al. 2010), but measurements of flow do not directly address current speed because flow is also a function of stream cross-sectional area. Humphrey and Stevenson (1992) manipulated current speed and nutrient concentrations in experimental stream channels to simulate a subscouring spate and concluded that spates could stimulate algal growth in nutrient-rich waters but inhibited it in nutrient-poor streams. To our knowledge, simultaneous effects of nutrient addition and current speed have not been tested.

We tested the hypothesis that the benthic algal biomass response ratio (BRR; the ratio of nutrient-addition to control treatment benthic algal biomass) to nutrient addition is positively related to current speed. Biomass losses to grazer consumption (Hillebrand 2009), dislodgement by grazers (Scrimgeour et al. 1991), autogenic sloughing driven by light and nutrient limitation within the periphyton layer (Biggs 1996, Peterson 1996), and shear and drag forces (Stevenson 1996, Biggs et al. 1998) can make detecting the combined effects of nutrient addition and current speed difficult. Several confounding and interacting influences could mask the effect of current-mediated nutrient availability on benthic algal biomass. Current speed can influence macroinvertebrate grazing (Opsahl 2003, Wellnitz and Poff 2006), and grazing (DeNicola and McIntire 1991, Rosemond et al. 1993, Pringle 1990, Darcy-Hall and Hall 2008) and current speed (Poff et al. 1990, Biggs and Hickey 1994, Biggs et al. 1998) can affect benthic algal composition, physiognomy, and indirectly, resistance to sloughing. Moreover, the relative effects of bottom-up enrichment and top-down grazer control on benthic algal biomass could vary with current speed, and benthic algal response to nutrient addition can vary among substrata (Pringle 1990). Therefore, we measured grazer abundance across current speeds and nutrient treatments, and we used pavers to standardize substratum effects. We standardized paver depth to account for potential interaction between light and current speed (DeNicola and McIntire 1990).

Methods

Experimental design

During the dry season between July and September 2008, we conducted a field experiment on gravel-pebble substrata along a 19.3-km reach of the 7th-order Daly River in the Australian wet/dry tropics, downstream of Oolloo Crossing at lat 14°04'10"S, long 131°15'02"E. Dry-season discharge originated from groundwater inputs upstream of the study reach,

and was measured by the Department of Natural Resources, Environment, The Arts and Sports (NRETAS). The study reach was 50 m wide, oriented southwest-northeast, and had an open canopy. During the dry season, the river is oligotrophic based on the classification system of Dodds et al. (1998) with total P and total N concentrations of 5 to 10 µg/L and 60 to 190 µg/L, respectively (Townsend and Padovan 2005).

We designed an unreplicated experiment with 2 factors: current speed and nutrient addition. We chose 6 sites to cover a range of current speeds (<2–98 cm/s), and the nutrient factor comprised a control and 1 nutrient-addition treatment. The experiment comprised 5 current speed categories: 0, 27, 48, 78, and 98 cm/s. We aggregated data from 2 sites post hoc because their current speeds, which were ~27 cm/s at the end of the experiment, did not differ significantly (*t*-test, *n* = 16, *p* = 0.26).

On July 8, we deployed 2 sets of 16 unglazed clay pavers (11.4 × 11.1 × 3.8 cm), arranged in a 4 × 4 matrix at each site in water 48 to 70 cm deep. At 4 of the 6 sites, we placed 2 sets of pavers adjacent to each other, 2 to 10 m apart and perpendicular to the current, and then assigned 1 set randomly to the control treatment. Otherwise, we arranged the pavers 20 to 200 m apart longitudinally and assigned the upstream set to the control treatment. We confirmed hydraulic isolation of the adjacent paver sets by observing colored vegetable dye released between the 2 sets. We allowed periphyton to colonize pavers and become established for 5 wk before nutrient addition.

On 12 August, we added nutrients to the treatment paver sets in the form of commercially available slow-release fertilizer pellets (Osmocote Plus; Scotts Australia P/L, Bella Vista, Australia). The pellets were 15% N and 4.4% P by mass, with a soluble N (NO₃⁻ and NH₄⁺) to soluble P molar ratio of 10:1. Other nutrients by mass were 10% K, 2.5% S, 0.4% Fe, and 0.2% trace elements. Addition of both soluble N and P permitted growth of algal species that were limited by either nutrient because algal assemblages are typically responsive to both nutrients (Francoeur 2001). We did not add Si because concentrations in the streams are ~11 mg/L (Townsend et al. 2012) and are unlikely to limit diatom growth. At 4 sites, we filled mesh bags (2 mm mesh, 14 × 8 cm) of plastic shade cloth with either 1.5 kg of fertilizer pellets or similar-sized plastic beads. We deployed a bag 20 cm upstream of each of the 4 upstream pavers at the flowing sites. At the quiescent site, we distributed fertilizer pellets between the pavers rather than placing them in bags.

The addition of fertilizer was not expected to produce a large (e.g., 10×) increase in nutrient

concentration but instead to increase ambient nutrient concentrations marginally. We used a laboratory experiment to evaluate nutrient release from the pellets by measuring the mass released over 30 d at 28°C into 10 L of distilled water. The release rate was linear, and approximate daily rates were 950 mg NH₄-N, 900 mg NO₃-N, and 230 mg PO₄-P for each 1 kg of fertilizer pellets. If these nutrients were released daily into 1-L volumes passing at a current speed of 25 cm/s and diluted 20×, this rate would result in an additional 3 µg/L of NH₄-N, 3 µg/L NO₃-N, and 1 µg/L PO₄-P, respectively, in the water passing over the pavers. We deployed the same quantity of fertilizer pellets at each site, but flow through each bag of fertilizer was unlikely to be equal and probably increased in proportion to current speed. The additional nutrient concentrations would have been lower with increased current speed, though nutrient load would have been theoretically equal, assuming the same nutrient release rates.

We conducted nutrient addition over a 5-wk period. We replaced the pellets with new material after 3 wk at the flowing sites and deployed more pellets at the quiescent site. We recovered the pavers between 16 and 21 September. At each site, we randomly selected 3 pavers for analysis of chlorophyll *a*, 3 for analysis of macroinvertebrates, and 1 paver for analysis of algal taxonomy. The other pavers were used for analyses not reported here. We treated the replicate pavers as subsamples of each current-speed–nutrient-addition treatment combination.

Measurements and sample analyses

During the experiment, we collected water-quality samples and made measurements 30 cm toward the center of the paver set from each corner of each paver set. We collected samples on 3 occasions: 1) immediately before deploying fertilizer and control bags, 2) immediately before replacing fertilizer pellets, and 3) when the pavers were recovered. We measured temperature, pH, and conductivity with a Hydrolab Quanta water-quality sonde (Hach Hydromet, Loveland, Colorado), and turbidity with a Hach 2010P meter.

We collected water samples for analysis of NH₄⁺, oxidized N (NO₃⁻ and NO₂⁻), and filterable reactive P (FRP), filtered the samples in the field through a 0.45-µm membrane, and stored them frozen until analysis by standard methods (APHA 1998). These were phenate colorimetric method for NH₄-N (APHA 1998 method 4500D), the automated Cd method for NO₃-N and NO₂-N (method 4500-NO₃⁻ F), and the automated ascorbic acid reduction method for FRP (method 4500-P F), each with a detection limit of 1 µg/L.

We measured depth (D) and current speed when the sites were selected and the pavers deployed in July with an Ott propeller current meter (model C31, Ott Hydromet, Kempton, Germany), and in September with a FlowTracker handheld acoustic doppler velocimeter (SonTek/YSI, San Diego, California) during paver recovery. We made 4 measurements at each control and nutrient-addition set of pavers, 30 cm inward from the each corner of the paver sets, at a depth of 0.4D from the river bed, which approximates the depth-averaged current speed (Gordon et al. 2006).

We measured 4 profiles of photosynthetically active radiation (PAR) in pools along each reach in July and September with a Li-Cor 188B submersible scalar bulb (Li-Cor, Lincoln, Nebraska), and calculated the PAR attenuation coefficient from the slope of the regression of depth (independent variable) and ln(PAR). This value is expressed as the euphotic depth (m), the depth to which 1% of PAR immediately below the surface penetrates.

We collected chlorophyll *a* (Chl) samples by scraping all of the exposed (unburied) sides of the paver with a paint scraper into a specimen jar, and we measured the paver area sampled (0.021–0.029 m²). We contend that the large area sampled (~0.075 m²) for each test category was a methodological strength of the experiment because it ensured that sparse tufts of filamentous algae were sampled. We stored the Chl samples immediately on ice, then froze them until analysis in the laboratory. We thawed the sample, ground it with mortar and pestle, and analyzed it by spectrometry in a 90% acetone solution using standard method 10200H (APHA 1998), and corrected for phaeophytin.

We collected macroinvertebrate samples by scraping pavers with a paint scraper and rinsing them with distilled water into a 500-µm plankton net. We preserved all macroinvertebrates in the field in 70% ethanol. We identified all Bivalvia and Insecta to family. We identified all other macroinvertebrates to class under 60× magnification. We analyzed data only for macroinvertebrate families or classes that were predominantly composed of grazers by summing the abundances of Chironomidae, Empididae, Caenidae, Baetidae, Leptophlebiidae, Leptoceridae, Elmidae, Nymphulinae, Gastropoda, and Oligochaeta (Merritt and Cummins 1996). This categorization omitted some families, such as such as Ecnomidae, that have a few grazer species and included some families with a few nongrazer taxa.

We collected samples for algal taxonomy by the method described for Chl and preserved samples for algal taxonomy with Lugol's iodine. We shook the

samples gently for 30 s to homogenize them before subsampling. We examined the subsample under 400 \times magnification using a Lund cell on a Zeiss Axioskop microscope (Carl Zeiss, Göttingen, Germany) and the method of Hötzel and Croome (1999). We identified most nondiatom algae at the species level. When we were unable to identify a taxon with certainty, we provided it with a genus taxon number. Many genera were represented by a single individual, which precluded the need for further taxonomic identification. We did final taxonomic identification of diatoms from subsamples treated in dilute HCl and H₂O₂ and viewed under a differential phase contrast microscope at 1500 \times magnification with immersion oil (Townsend and Gell 2005). We examined 40 transects across the cell for each sample and ensured that ≥ 150 algal units (individuals, filaments, or colonies) of the 5 most frequently occurring taxa were enumerated to yield a counting precision of $\pm 16\%$ or better for these taxa. We counted the number of cells/algal unit, and we counted ≥ 700 units/sample. We measured the dimensions of each taxon in each sample and applied standard geometric formulae to calculate biovolume. We calculated total sample biovolume from the product of the taxon cell concentration and biovolume. We identified most taxa to genus level.

Statistical analyses

The experimental design was unreplicated, so we could not use 2-factor analyses of variance (ANOVAs) to test the effects of current speed and nutrient addition on algal biomass or grazer abundance. Instead, we used 2 sets of 5 individual *t*-tests, each set Bonferroni adjusted ($\alpha = 0.01$) for multiple tests to limit the experiment-wide error (Sokal and Rohlf 1998), to compare nutrient-addition and control treatment algal biomass and $\log(x)$ -transformed grazer abundance for each current-speed category. We also ran paired *t*-tests (Zar 1999) to compare differences between control and nutrient-addition treatment means for algal biomass and grazer abundances. We used Mann–Whitney nonparametric tests to compare water-column nutrient concentrations between control and nutrient-addition treatments because the data were not normally distributed.

We calculated the biomass response ratio (BRR) by the dividing the mean biomass (as Chl) of the 3 nutrient-addition subsamples by the mean of the control subsamples for each current-speed category. We tested the relationship between the BRR and current speed with linear regression analysis.

We examined algal and grazer taxonomic composition between nutrient-addition and control treatments

and among current-speed categories with nonmetric multidimensional scaling (NMDS) ordinations (PRIMER, version 6.1.12; Clarke and Gorley 2006) on Bray–Curtis similarity matrices of algal relative biovolumes and mean grazer relative abundances. We $\sqrt{(x)}$ -transformed the data to reduce the weight of dominant taxa in the analysis, but the domination of both data sets by a few taxa made these analyses insensitive to transformation (except a presence–absence transformation). We excluded algal taxa with relative biovolumes that did not exceed 1% in any 1 sample from the algal ordination. We used analyses of similarity (ANOSIM) to compare algal and grazer assemblages between nutrient-addition and control treatments.

Results

Discharge declined at an almost linear rate over the 10-wk duration of the experiment from 34 m³/s to 25 m³/s and the water level fell 18 cm with no disturbance from storm runoff events. In September, current speeds measured at the 5 sites averaged <2 cm/s, 27 cm/s, 48 cm/s, 78 cm/s, and 98 cm/s (Fig. 1A) and were 8 to 15% lower than July current speeds with the same relative differences between sites. Most of the reduction in flow over the study was caused by reduction in cross-sectional area. Mean current speeds did not differ between nutrient-addition and control treatments for any current-speed category (*t*-tests, all $p > 0.01$). Turbidity was low (1.8–2.9 NTU) during the experiment, and the euphotic depth, which averaged 8.20 ± 0.48 m and 8.04 ± 0.26 m in July and September, respectively, exceeded paver depths by a factor of ~ 15 .

The waters were warm with temperatures that increased from 25°C in July to 31°C in September, alkaline (pH 7.8), and averaged 560 $\mu\text{S}/\text{cm}$. Concentrations of soluble N and P were low and similar among sites in September at the end of the study (Table 1). Before we began the fertilizer treatment, median concentrations were 1 $\mu\text{g}/\text{L}$ NO₃-N, 9 $\mu\text{g}/\text{L}$ FRP, and <1 $\mu\text{g}/\text{L}$ for NH₄⁺ and NO₂⁻. During the experiment, median concentrations in control and nutrient-addition treatments did not differ (Mann–Whitney tests, all $p > 0.05$). The ranges of NH₄⁺ and NO₃⁻ concentrations were higher in nutrient-addition than control treatments (Table 1). In $\sim 10\%$ of samples, NH₄⁺ and NO₃⁻ concentrations were >6 $\mu\text{g}/\text{L}$, but FRP concentrations were not similarly high, probably because of the relatively low P content of the fertilizer.

Algal biomasses spanned 2 orders of magnitude (0.8–22.9 mg Chl/m²) and were within the range

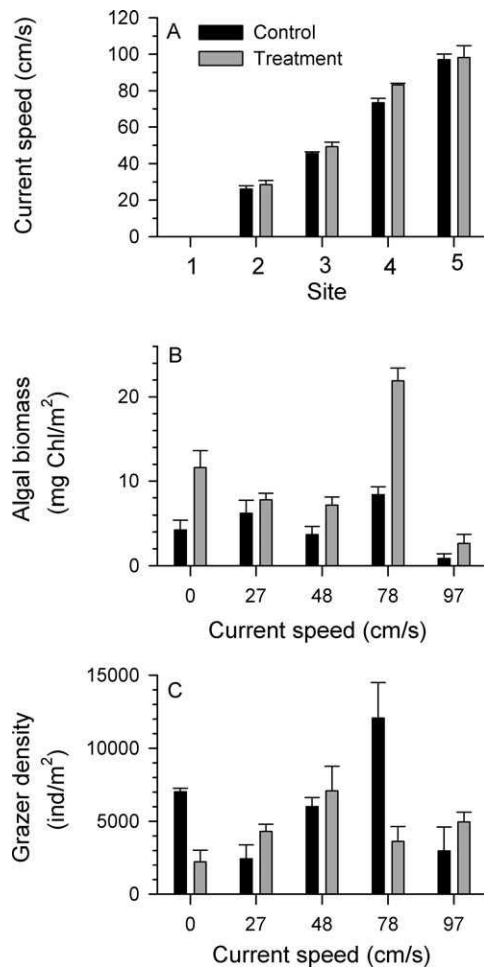


FIG. 1. Mean (± 1 SE) current speed at each site (A), and benthic algal biomass (as chlorophyll *a* [chl]) (B) and grazer density (C) for each current speed. Ind = individuals.

reported for *Spirogyra* in the Daly River (Townsend and Padovan 2005). On average, biomasses were 130% higher in nutrient-addition than in control treatments (paired *t*-test, $n = 5$, $p = 0.004$; Fig. 1B). BRRs for all current speeds were >1 and increased linearly between 27 and 98 cm/s range ($r^2 = 0.99$, $p = 0.006$; Fig. 2), whereas the ratio at the quiescent site

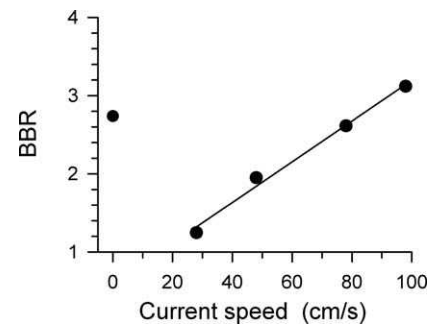


FIG. 2. Regression for mean algal biomass (as chlorophyll *a*) response ratio (BRR) to nutrient addition as a function of current speed. Linear regression: $BRR = 0.026 (\pm 0.0018 \text{ SE}) \times \text{current speed (cm/s)} + 0.59 (\pm 0.12 \text{ SE})$, $r^2 = 0.991$, $p = 0.005$. The <2 cm/s data point was excluded from the regression.

(2.7) was similar to ratio at the 78 cm/s site (2.6). Absolute differences between the control and treatment biomasses for each current speed showed no linear relationship with current speed, being highest and lowest for the 78 cm/s and 97 cm/s current speeds respectively (Fig. 1B). The low biomasses for the control and treatment at the 97 cm/s site are attributable to sloughing losses (Townsend and Padovan 2005).

Benthic algal biovolume was dominated by 3 filamentous chlorophytes in nutrient-addition and control treatments and among current speeds (Fig. 3A). These taxa were *Mougeotia* sp. (cell length $\sim 250 \mu\text{m}$), *Oedogonium* sp. (cell length $\sim 80 \mu\text{m}$), and *Spirogyra* (cell lengths $\sim 200\text{--}300 \mu\text{m}$) (Townsend et al. 2008). Their average biovolume relative abundances were 10%, 39%, and 47%, respectively. The domination of these chlorophytes was a result of their large cell volumes, which exceeded the median biovolume of $90 \mu\text{m}^3$ by a factor of 600 for *Mougeotia* and 2000 for *Oedogonium* sp. and *Spirogyra*. Cyanobacteria were a minor component of the assemblage ($<1.5\%$ biovolume) except at the 98 cm/s site where they contributed $\sim 8\%$ to the total biovolume and were dominated

TABLE 1. Concentrations of nutrients ($\mu\text{g/L}$) above the pavers in the control and nutrient-addition treatments for the 5 current-speed categories. $n = 48$ for each data set. FRP = filterable reactive P.

Nutrient	Treatment	Minimum	Median	92 nd percentile	Maximum
NH_4^+	Control	<1	<1	1	1
	Nutrient-added	<1	<1	19	23
NO_2^-	Control	<1	1	1	1
	Nutrient-added	<1	1	1	1
NO_3^-	Control	<1	1	2	3
	Nutrient-added	<1	2	23	25
FRP	Control	5	9	12	14
	Nutrient-added	8	10	12	17

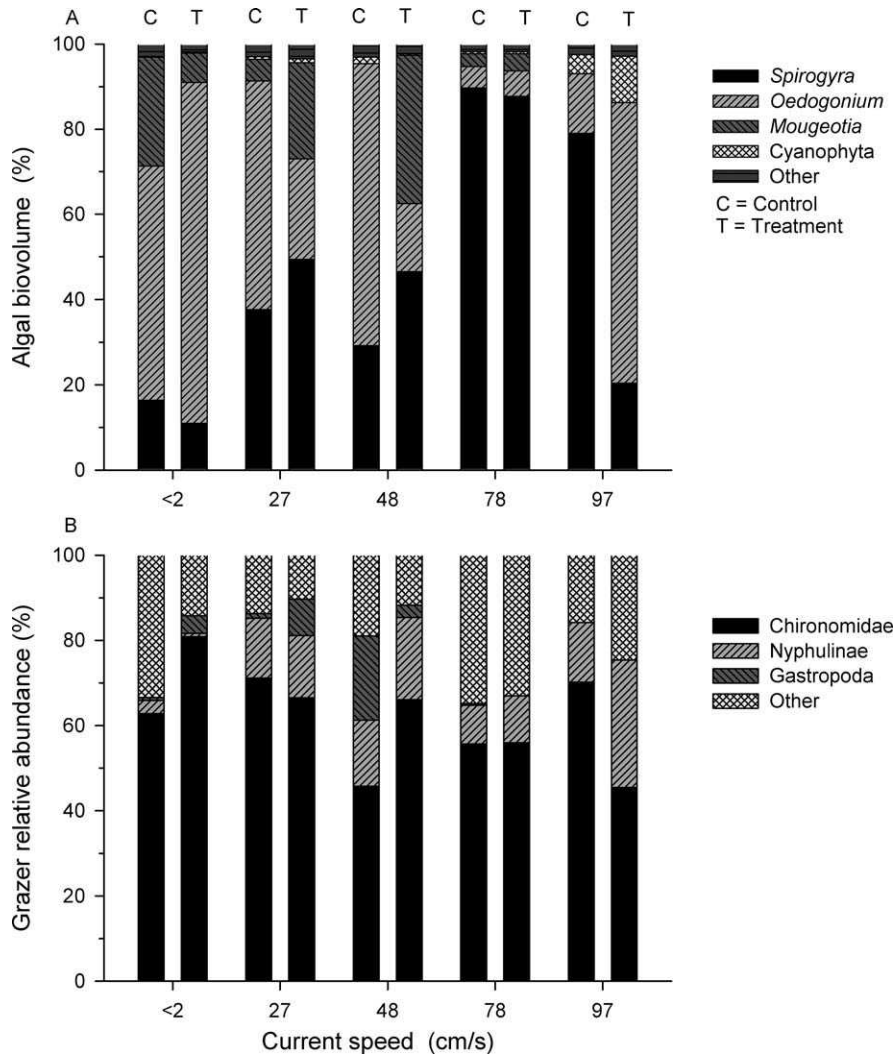


FIG. 3. Relative abundance of benthic algal taxa (based on biovolume) (A) and invertebrate grazers (based on counts) (B).

by *Cylindrospermum cf. licheniforme*. Diatoms were a minor component of the biovolume (<2.3%) but constituted 68% of the 218 taxa identified. Fifty-five percent of diatom taxa were observed only once and 32% were observed only twice. Ordination of the biovolume data revealed no pattern of algal assemblage composition across the range of current speeds or between nutrient-addition or control treatments (Fig. 4A). Assemblage composition did not differ between nutrient-addition and control treatments (ANOSIM, global $R = -0.12$, $p = 0.77$).

Grazer densities ranged between 1500 and 16,700 individuals (ind)/m² and did not differ between control and nutrient-addition treatments (paired t -test, $p > 0.05$) or among current-speed categories (t -tests, all $p > 0.01$) (Fig. 1C). Chironomids dominated the grazer macroinvertebrate community in all current speeds and nutrient treatments, whereas gastropods and

Nymphulinae larvae differed most among current speeds (Fig. 3B). Ordination of mean relative abundances revealed no gradient associated with current speed (Fig. 4B) or differences in grazer assemblages between nutrient-addition and control treatments (ANOSIM, global $R = -0.08$, $p = 0.78$), but our classification scheme may have masked differences among higher taxonomic levels.

Discussion

We demonstrated a bottom-up effect of nutrient addition on benthic algal biomass that varied in strength across current speeds. Nutrient addition produced positive BRRs across all current speeds tested, with an average ratio of 2.3, which is almost equal to the average for the 237 nutrient addition experiments analyzed by Francoeur (2001), although 2

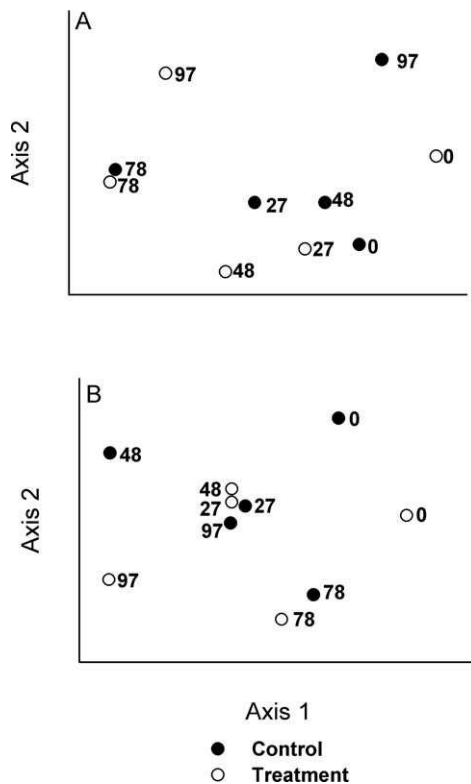


FIG. 4. Nonmetric multidimensional scaling ordination plots for algal relative biovolume (A) and invertebrate grazer invertebrate abundance (B). Numbers next to symbols show current speed (cm/s).

of the 5 ratios (1.2, 3.1) exceeded the 95% confidence interval for the meta-analysis.

In our study, the positive response to nutrient addition occurred when ambient nutrient concentrations were generally low, probably because of the slow release of nutrients from the pellets, rapid uptake by periphyton, and nutrient-limited filamentous macroalgae (Townsend et al. 2012) and phytoplankton (Townsend et al. 2008), but phytoplankton are a minor component of the river's dry season primary producer biomass (Webster et al. 2005). High NO_3^- and NH_4^+ concentrations suggest infrequent pulses of enriched water from the fertilizer bags possibly caused by uneven flow through the bags and release of pockets of accumulated dissolved nutrients. Nutrient delivery regime can affect benthic algal response. Continuous low dosage rates are favorable to *Spirogyra* growth, whereas other algae can respond preferentially to short high-nutrient-concentration pulses (Borchardt et al. 1994). The near-detection-limit nutrient concentrations and just 2 sampling dates limit any conclusion about the nutrient delivery regime, but a continual low dosage with occasional enriched pulses is the most likely situation. Worm

et al. (2000) recommended frequent monitoring of nutrient concentrations released from fertilizer pellets. We inferred the effectiveness of our enrichment method from the higher algal biomasses in the nutrient treatments.

Over the range of 27 to 98 cm/s, the BRR increased linearly with current speed, supporting our hypothesis. This result is consistent with the prediction that increased current speed will reduce the thickness of the DBL and, therefore, will produce higher rates of nutrient mass-transfer across the layer to the cell membrane to support growth and biomass accumulation (Riber and Wetzel 1987). The results suggest that even at a current speed of 98 cm/s, the thickness of the layer had apparently not reached a saturating current speed that marked the shift between mass-transfer and kinetic control of nutrient uptake (see Sanford and Crawford 2000). The results of our field experiment are in agreement with those of a laboratory experiment conducted by Larned et al. (2004), who showed linear relationships between algal nutrient uptake rates and near-bed velocities when nutrient mass-transfer limited growth.

The current-speed-response-ratio regression predicted a BRR <1 for current speeds <18 cm/s when extrapolated to the x -axis. This value is improbable in these nutrient-depleted waters. This result may have been caused by our use of depth-averaged current speed as a surrogate for the hydraulic conditions at the DBL and could imply a nonlinear relationship as current speeds approach 0 cm/s. In contrast, the extrapolated regressions calculated by Larned et al. (2004) extended to the positive nutrient-uptake y -axis when near-bed current speed was 0 cm/s.

Several factors contributed to the pronounced relationship between current speed and BRR. These were the oligotrophic conditions, the form of nutrient addition, the dominance of filamentous chlorophytes, and the inferred weak top-down effect of grazing between the control and treatment. Under the oligotrophic conditions of this experiment, nutrient uptake is most likely to be controlled by mass-transfer (Larned et al. 2004) and nutrient concentrations below saturation because nutrient uptake can decline with increasing concentration (Horner et al. 1990). No relationship between current speed and biomass was reported by Biggs et al. (1998) at high concentrations (48 $\mu\text{g/L}$ soluble reactive P). Positive BRRs at the low nutrient concentrations do not support the concept of a threshold of nutrient response posited by Lewis and McCutchan (2010). Thus, our results also serve to emphasize the need to consider current speed explicitly as a covariate when nutrient-addition experiments are designed and analyzed.

TABLE 2. Theoretical nutrient availability to epilithic benthic algae for combinations of nutrient concentration and current-speed increases and decreases relative to a reference current speed and nutrient concentration. Nutrient limitation and no other effects such as sloughing or grazing are assumed. A positive sign indicates increased nutrient availability, and negative sign indicates decreased nutrient availability. Opposing signs are not expected to cancel each other and show only that the effects are opposing. The 1st sign of the pair shows effects of variation in nutrient concentrations, and the 2nd sign shows effects of variation in current speed.

Change relative to reference	Decreased current speed	Increased current speed
Increased nutrient concentrations	+/-	+/+
Decreased nutrient concentrations	-/-	-/+

The form of nutrient addition can affect algal nutrient uptake. Addition of soluble nutrients favors growth of protruding filamentous algae, which were dominant in our study, whereas addition of nutrients in their organic forms favors algae embedded within the periphyton (Pringle and Bowers 1984). The pronounced relationship between current speed and the BRR underpinning our results might not have been evident had benthic algae been embedded in a periphyton matrix rich in nutrients, which would have provided an alternative nutrient supply.

Algal species vary in their nutrient requirements for growth (Borchardt 1996, Biggs et al. 1998). This potentially confounding factor was negated to a large extent by the dominance of filamentous chlorophytes across current speeds and nutrient treatments. In contrast to responses of benthic algae to eutrophication (see Smith 2003), nutrient addition was not sufficient to cause a major shift in algal composition.

Strong top-down effects of grazers on stream benthic algal biomass have been found in many experimental and observational studies (Feminella and Hawkins 1995, Hillebrand 2009) and this grazer effect could confound a nutrient-addition effect. We contend that grazing rates during our experiment were probably similar between the nutrient addition and control treatments for each current-speed category but differed among current speeds as indicated by the differences in abundance and community composition. The similarity in algal assemblages between control and nutrient-addition treatments suggests no strong interaction between grazing and nutrient addition. Macroinvertebrate grazing can favor growth of less palatable algal taxa, such as filamentous chlorophytes (Rosemond et al. 1993), but in our experiment, these taxa dominated both control and nutrient-addition treatments. The high C:N:P ratio of filamentous algae, which averages 1700:88:1 for *Spirogya* in the Daly River and its tributaries (Townsend et al. 2008), may have constrained grazing rates. Top-down grazer effects tend to increase with increasing algal biomass (Hillebrand 2009), but this effect would have reduced our ability to detect

bottom-up nutrient addition effects. These considerations suggest that the nutrient-enrichment effect was greater than the grazing effect on algal biomass across current speeds.

At the quiescent site, nutrient enrichment of the surrounding waters could be expected to depend on molecular diffusion within the water body if truly stagnant conditions existed and mass transfer occurred across a thicker DBL than at the flowing sites. However, the quiescent site was connected to the main river channel and probably experienced diurnal currents akin to those found in the side arms of lakes (Monismith et al. 1990). Moreover, movement of fish, turtles, and crocodiles through the site would have mixed the waters. We observed a large crocodile mud-slide on the bank a few meters from the quiescent site. Thus, although current speed was not measurable during field trips, mixing and exchange with the surrounding waters probably occurred. The most likely explanation for the high BRR at the quiescent site was that higher nutrient availability relative to the flowing sites was not detected as high concentrations because of rapid uptake rates by both benthic algae and phytoplankton in the water column. The different physical conditions at the quiescent and flowing sites provide a rationale for the exclusion of the quiescent site from the regression analysis of current speed and BRR.

In conclusion, our results provide empirical evidence that a positive linear relationship exists between current speed and the algal BRR to nutrient addition, when filamentous chlorophytes dominate and oligotrophic conditions prevail. Current-mediated bottom-up control of nutrient availability for benthic algal uptake contributes to the regulation of benthic algal biomass on an epilithic substrate, and more broadly, to the patchiness of in-stream nutrient dynamics (Pringle et al. 1988), though several mitigating factors, such as algal composition and physiognomy, grazing, and physical losses, could mask or reduce this effect.

Combinations of nutrient-concentration and current-speed variations produce a range of theoretical

scenarios for the availability of nutrients to benthic algae (Table 2). These scenarios, underpinned by current-mediated nutrient availability, can either reinforce or counter the manifestation of these 2 determinants of benthic algal ecology. The effect of nutrient addition or pollution on riverine benthic algal biomass expressed as a BRR is greatest when current speeds are high (Table 2), but the absolute increase in biomass may be small (as occurred at the 98 cm/s site in our study; Fig. 1B) because of high sloughing losses (see Stevenson 1996). Flow regulation that reduces current speed, for example indirectly through water extraction during river baseflow, would reduce nutrient availability. Our study highlights the potential significance of current-mediated nutrient availability for responses to the anthropogenic impacts, eutrophication and flow regulation, and the patchiness of nutrient-driven bottom-up control of benthic algal biomass.

Acknowledgements

This manuscript was completed with the support of the Tropical Rivers and Coastal Knowledge (TRaCK) research hub. TRaCK receives major funding for its research through the Australian Government's Commonwealth Environment Research Facilities initiative, the Australian Government's Raising National Water Standards Program, Land and Water Australia, the Fisheries Research and Development Corporation, and the Queensland Government's Smart State Innovation Fund. We thank Julia Schult for preparing the mesh bags and running Chl analyses, and Danielle Warfe and Dominique Long for field assistance. The manuscript was much improved with the comments from Associate Editor Dean DeNicola and 2 anonymous referees.

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Received: 22 November 2011

Accepted: 15 August 2012