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Research Article

Catchment urbanization increases benthic microalgal biomass in streams under controlled light conditions

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Abstract. Stormwater from urban land degrades aquatic ecosystems. Nutrients, light and flow regime affect the development of benthic microalgae (microphytobenthos), and all are affected by urban stormwater. The relative influence of these factors on microphytobenthos is unknown and is largely untested. This study investigated the effect of urbanization, controlling for irradiance, on the development of stream microphytobenthos assemblages. Three light levels were achieved (two were comparable) in four streams of different catchment urbanization. Microphytobenthos assemblages were sampled fortnightly from each stream over 79 days in winter. Biomass (chlorophyll *a,* pheophytin and cell density) increased with catchment urbanization. Light only affected biomass in the more urban streams and scour may have affected microphytobenthos assemblages in the most urban stream. Each stream had distinct assemblages, although time and light had no apparent effect on their composition. Physiological analysis suggested that the microphytobenthos was potentially light-limited in all four streams. However, light limitation was overridden by nutrient limitation in the least urbanized streams. The alleviation of nutrient limitation in one stream under the highest light treatment was attributed to microphytobenthos having sufficient energy to support active uptake of nutrients. Light did not drive differences in microphytobenthos biomass among the four study streams; differences were due to other factors affected by urbanization, most likely nutrient enrichment. To minimize the risk of algal blooms in urban waterways, reducing eutrophication should be a higher management priority than limiting irradiance.

Key words. Microphytobenthos; stormwater; watershed; fluorescence; Melbourne; Australia.

Introduction

The expansion of urban land is a growing threat to the world's freshwater ecosystems (Malmqvist and Rundle, 2002). The impacts of urbanization (defined as an increase in the extent and/or density of urban areas) on streams, in particular, are severe and complex (Walsh et al., 2005b), and are most commonly dominated by impacts from urban stormwater runoff. Stormwater affects streams by diffusely delivering a complex mix of pollutants, increasing the frequency and intensity of high flow events and causing an increase in sediment supply and bankfull discharge that often results in widening and incision of channels

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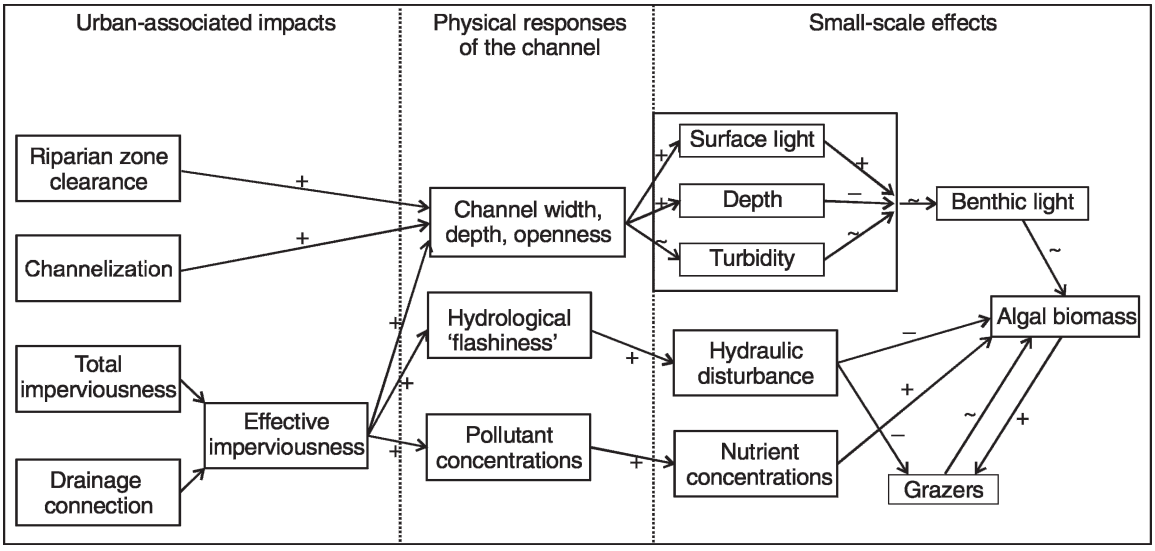

Figure 1. Conceptual model of urban-associated impacts on stream channels and the resulting primary factors hypothesized to be affecting benthic algal biomass in the study streams. Positive and negative effects on each attribute are indicated, and where the effect resulting from urban impacts are thought to be near neutral they are indicated by ~. After Taylor et al. (2004) changes to channel morphology and water turbidity are hypothesized to result in little change to benthic light climate. The most likely primary urban-associated mechanism explaining observed increases in algal biomass is increased nutrient concentrations arising from stormwater inputs.

(Paul and Meyer, 2001). Such catchment-scale impacts are often compounded by local-scale impacts such as clearance of riparian vegetation, channelization and other in-stream engineering works that widen the channel, all of which can elevate irradiance levels at the stream surface (Fig. 1).

Increased irradiance, together with stormwater-derived nutrients, may lead to increased biomass of benthic microalgae, or microphytobenthos (MPB), in streams impacted by urbanization (Fig. 1). However these stimulatory effects may be countered by increased flow disturbance (shear stress and scouring), light attenuation through increased turbidity or channel depth, increased toxicity from contaminated sediments (Fig. 1; Paul and Meyer, 2001), or even by direct, deliberate application of algicides into waterways (Grimm et al., 2005).

Not surprisingly, therefore, the limited studies that have investigated patterns of stream MPB biomass in response to urban land use have reported contrasting patterns. Roy et al. (2005) found that algal biomass was poorly correlated with urbanization but was higher in open reaches compared to more shaded, forested reaches. Similarly, patterns of MPB biomass were poorly explained by urban land use in Pennsylvania (USA) streams (Hession et al., 2004), or in streams of western Georgia, USA (B. Helms and J. Feminella, Auburn University, personal communication). However, algal abundance in Minnesota (USA) streams was strongly correlated with urban land use (Richards and Host, 1994), and in Melbourne, Australia, streams of urbanized catchments supported higher algal biomass, and demonstrated at least partial release from nutrient limitation that was observed in rural streams (Chessman et al., 1992).

In a subsequent study of Melbourne streams, MPB biomass was positively correlated with catchment urban density, which was most strongly explained by effective imperviousness (the proportion of catchment covered by impervious surfaces with direct sealed drainage connection to the stream) (Taylor et al., 2004; Walsh et al., 2005a). Effective imperviousness provides a surrogate for stormwater impact, which is distinct from sewage impact; in Melbourne, the sanitary sewerage system is separate from the stormwater system.

MPB growth in streams may be limited by several factors, including nutrients (Borchardt, 1996), light (Hill, 1996), physical (flow-related) disturbance (Peterson, 1996) and grazing (Steinman, 1996). All of these factors may be affected by catchment urbanization (Fig. 1) but their relative influence on MPB remains unknown and is largely untested. Microalgae, particularly diatoms, reflect ecosystem health (see Whitton and Kelly 1995; Winter and Duthie 2000). Nuisance algal growth can result in blooms that degrade stream condition and some species may also be toxic (Anderson et al. 1998). The effective management of MPB in streams subject to urban impacts requires knowledge of the relative importance of each of these factors and their interactions, particularly in regions where MPB biomass and urbanization are positively correlated.

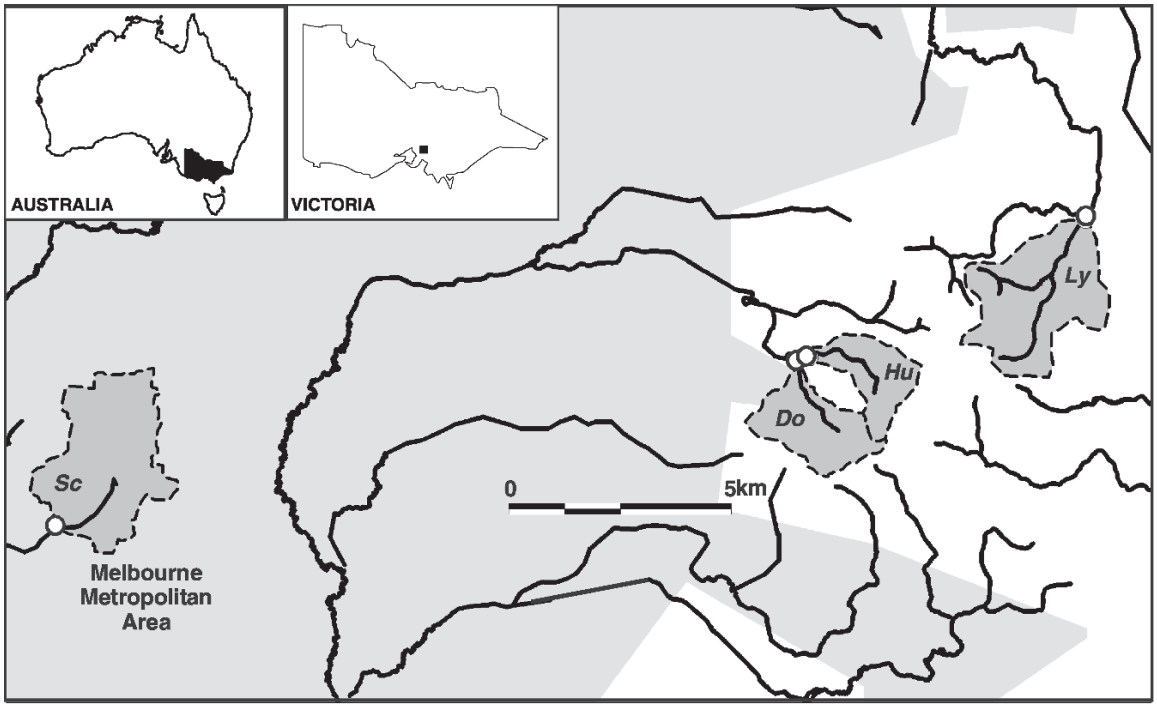

Figure 2. Catchment area and location of the four study streams (Sc, Do, Hu and Ly) relative to each other and the metropolitan region of Melbourne (shaded).

Taylor et al. (2004) posited that stormwater-derived phosphorus was the primary cause of increased MPB biomass in Melbourne streams. Benthic irradiance explained little of the variation in MPB in their study streams, being poorly correlated to the urban gradient, albeit spatially variable within each stream. In this study, we experimentally controlled benthic irradiance at 3 levels in a subset of streams studied by Taylor et al. (2004) to assess the relative effect of irradiance on MPB biomass, composition and physiology. In what we believe to be the first experiment of its kind, we tested if the response of MPB to benthic irradiance varied among streams of different catchment urbanization by controlling light levels. We use this experiment to discuss the likely effectiveness of riparian management to control MPB biomass by reducing irradiance in streams that receive increased nutrient loads from stormwater. Thus, if irradiance is the dominant influence on MPB assemblages, algal biomass could be modulated by controlling riparian vegetation cover and hence light availability for algal growth. However, such a strategy would not be relevant if irradiance is shown to be of little importance in limiting MPB assemblages.

Materials and methods

Site description

The study was conducted in four streams on a gradient of urbanization in the eastern suburbs and eastern fringe of Melbourne (Fig. 2). The increasing level of urbanization from Lyrebird Creek (Ly) to Hughes Creek (Hu) to Dobsons Creek (Do) to Scotchmans Creek (Sc) was indicated by an increase in effective imperviousness. This gradient of urbanization is referred to as the 'stream effect' or 'stream urbanization' in this paper. Nutrient levels, especially phosphorus, showed a positive correlation with effective imperviousness in the study streams (Table 1, Taylor et al., 2004). Methods for determination of catchment characteristics are described in Taylor et al. (2004). Catchments of a similar size, climate, soil and geology were chosen, and stream reaches that had similar light regimes were selected (based on data gathered in a pilot study). Differences in altitude caused slight differences in the median baseflow temperature of the streams (Table 1). However, temperature differences decreased towards the end of the experiment; the largest inter-site difference, between Sc and Ly, was 2.3 °C at the beginning of the study period and was 1.1 °C at the end. Such differences in temperature were deemed negligible.

Table 1. Catchment characteristics and general condition of the four streams. Values for water quality variables are median baseflow concentrations (source: Hatt et al., 2004). FRP, filterable reactive phosphorus; NOx, oxidised nitrogen (nitrate/nitrite); NH_3, Ammonia; TSS, total suspended solids.

	Stream			
	Scotchmans Creek	Dobsons Creek	Hughes Creek	Lyrebird Creek
Catchment characteristics				
Area (km^2)	8.1	3.7	2.8	7.3
Total imperviousness (%)	30.4	6.2	2.9	0
Effective imperviousness (%)	30.3	2.9	0.3	0
Dominant land use	Residential; some commercial	Forest; residential	Forest; residential	Forest
Stream characteristics				
Riparian vegetation	Trees and scrub native and exotic (deciduous); vegetation corridor patchy (0–3 m wide)	Trees mostly native; understorey and ground layer dominated by exotic weeds; vegetation corridor entire (1–3 m wide)	Eucalyptus canopy; tree ferns; dense, native ground layer and understorey; vegetation corridor entire (>5 m wide)	High Eucalyptus canopy; dense, native vegetation; vegetation corridor entire (>5 m wide)
Width (m)	4–8	1–2	0.5–1.5	0.5–1.5
Depth (m)	0.5–1.5	0.3–1	0.1–0.6	0.3–0.6
Median baseflow temperature during study period (°C)	10.6	9.4	9.7	9
FRP (mg L^{-1})	0.012	0.008	0.006	0.003
NOx (mg N L^{-1})	0.27	1.35	1.35	0.31
NH_3 (mg N L^{-1})	0.032	0.017	0.010	0.015
TSS (mg L^{-1})	4.2	11	16.5	38
Dominant substratum	Gravel	Rock and silt	Sand	Silt

Experimental design

In each stream, three blocks of three Perspex plates, overlain by filters to manipulate light transmission, were suspended using star pickets in a well-lit pool (36 plates in total). The three plates within one block were orientated parallel to the direction of flow, as some of the streams were too narrow for the blocks to be perpendicular to the flow direction (Fig. 3). The surface of the plates was within 10 cm of the water surface. The plates each contained 30 frosted glass slides, a commonly used artificial substratum in MPB experimental studies (Aloi, 1990). On each sampling date (6 collections made on odd-numbered weeks [1, 3, 5, 7, 9, 11]: 6 May-24 July 2002), the depth of water above the plates was measured, and five random slides were collected from each plate. A water sample was also collected at each site on each date for total suspended solids (TSS) determination using standard methods (Clesceri et al., 1998). Caught debris, if any, was removed from the plates and the filters were regularly cleaned with a brush to prevent fouling. The term 'time effect' used in this paper refers to the changes observed in the MPB samples over the 79-day duration of the field deployment.

The amount of light that each plate would receive in the absence of a filter was estimated by measuring the light climate at the water surface over the plate and was then corrected for attenuation through the water column. The surface light climate was estimated by analysing canopy photographs (taken at dawn directly above the algal plate blocks with a 180° hemispherical (fisheye) lens; (Canham, 1988; Canham et al., 1994; Asner et al., 1998) with the Gap Light Analyser software program (Frazer et al., 1999) (estimates were ground-truthed with an *in situ* light meter (Li-Cor Data Logger LI-1000 Q1 cosine corrected quantum sensor)). A coefficient for underwater light attenuation (c) was estimated by measuring light transmitted from a steady fluorescent light source through a 59 mm water column (of known TSS concentration) with a light meter (Li-Cor Data Logger LI-1000 Q1 cosine corrected quantum sensor) using the equation of Kirk (1983) (5 samples per stream). The mean in-stream attenuation over the study period was estimated using relationships among c, stream water level, TSS concentration and plate depths. A single value of c was applied for each stream to correct the estimated surface light over each plate for attenuation through water to the plate's measured depth.

Light intensity was manipulated with neutral density filters (photographic synthetic filters) that were positioned 20 mm over the plates. The filters gave three light intensity treatments in each block (100 %, 50 % and 12.5 % light transmission, including photosynthetically active radiation wavelengths (PAR; 400–700 nm)), which were ordered randomly

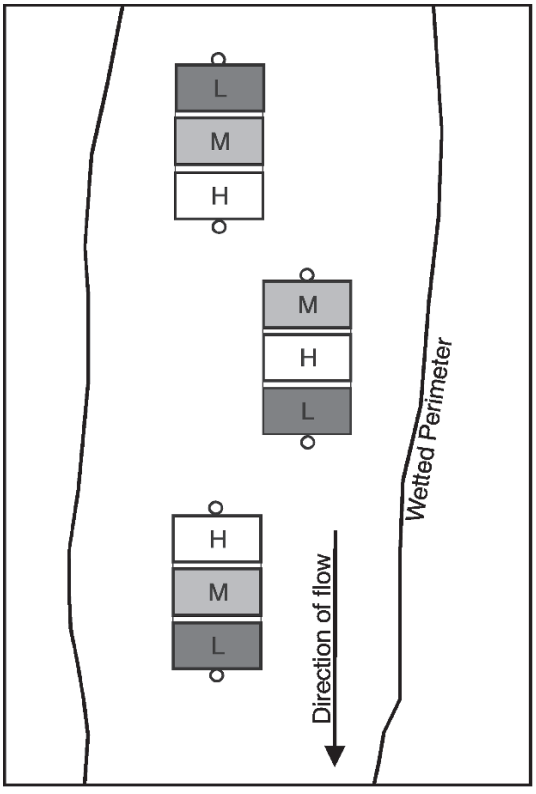

Figure 3. Example of arrangement of blocks in a stream. Low (L), medium (M) and high (H) light treatment plates were arranged randomly along each block.

with two levels of light that standardised the treatments across streams more closely (Fig. 4b). The medium-light treatment at Sc and Do was recoded as high-light, and a comparison was made between high- and low-light treatments for all streams. Only MPB under three levels of light were compared within each stream. The 'light effect' referred to in this paper denotes the influence of the three levels of PAR that were manipulated for this study.

Flow velocity was measured immediately in front of the plates, using a flow meter, 8 times during the experiment (5 cm diameter turbine head, number of revolutions per 20 seconds). Stream water level, temperature and nutrient concentrations were measured fortnightly in these four streams for 18 months in a concurrent study (Hatt et al., 2004), which encompassed the period of this study.

Determination of algal biomass

Algal biomass was represented by chlorophyll *a,* pheophytin and cell density. Chlorophyll *a* (Chl *a*) is a well-established proxy for estimating algal biomass and pheophytin is its breakdown product, used to estimate the proportion of moribund algal cells and/or detrital plant material in a sample (Romani and Sabater, 1999; Atwell et al., 1999; Gregor and Marsalek, 2004). Two slides collected from each

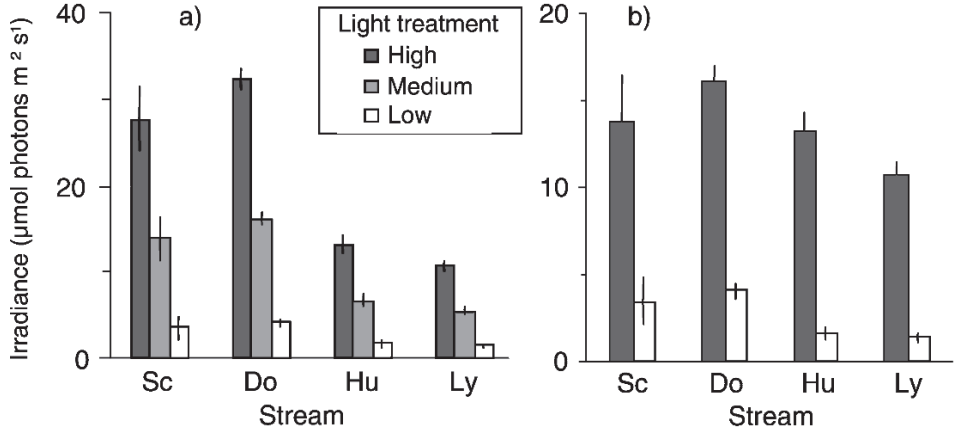

Figure 4. Mean incident light on algal plates in study streams based on water attenuation, canopy attenuation and neutral density filter treatments (a). three light treatment (b). two (recoded) light treatments. Mean incident light is the average irradiance reaching the nine algal plates in each stream during daylight hours (12 hours), consistent with studies in the literature. (Standard error bars for each of the streams (light regime determined for each block of three plates)).

along each block. Light intensity received by each nominal light treatment varied among the streams because of the different light environments (Fig. 4). As a result, effects of light and stream on algal biomass were analyzed in two ways: firstly, with the three levels of light as defined by filter density (High—H—, medium—M—and low—L—: Fig. 4a), and secondly,

plate each sampling trip (frozen at –80 °C for 2–10 weeks) were used to determine the amount of Chl *a* and pheophytin. Pigments were extracted overnight at 2°C with 90 % acetone, the samples were then pulse-sonicated (Sonifier® Cell Disruptor B30; 30 seconds), centrifuged (Sorvall RT6000 Refrigerated Centrifuge; 15 minutes at 3000 revs per minute) and the super-

natants analysed with a Cary 50 Spectrophotometer using the method of Lorenzen (1967). Cell density was determined from proportional counts scaled in accordance with the corresponding dilution factor when examining assemblage composition, as detailed below.

Determination of assemblage composition

One slide collected from each plate on each date was used to determine assemblage composition. Each sample was fixed by adding 250μL of Lugol's iodine. Algae were removed from the slides by sonication for 5 minutes in 10 mL of stream water in a waterbath sonicator (Transtek Systems Soniclean), pulse-sonication for up to 10 seconds at the lowest output intensity (1/10; Sonifier ® Cell Disruptor B30) and scrubbing with a small brush. A pilot study showed that sonication removed the cells from the slides without damage. A minimum of 100 cells were counted for each sample using a Sedgwick-Rafter cell and a Leica Leitz DM IRB microscope at 1000 times magnification. Cells were classified into six taxa – pennate diatoms and centric diatoms (Bacillariophyta), green filamentous algae and single green cells (Chlorophyta), and coccoid blue-green algae and filamentous blue-green algae (Cyanobacteria). Red algae (Rhodophyta) were not encountered in this study.

Physiological analyses

The degree of photoacclimation (phenotypic adaptation to changing irradiance levels; Atwell et al., 1999), physiological status and *in situ* production of MPB can be inferred from photosynthesis-irradiance (P-I) characteristics (Light and Beardall, 2001). Chlorophyll fluorescence analysis was used in this study to gauge the photosynthetic competence and physiological state of the MPB (Reynolds 1984; Baker and Horton 1987; Schreiber et al., 1994).

Two slides collected from each plate were stored in 3 mL of stream water, kept on ice and, on return to the laboratory, the photosynthetic characteristics of the algae were measured with a Walz Underwater Pulse Amplitude Modulated fluorometer (PAM). The PAM fibre optic sensor was positioned 5 mm above each slide and for each slide, two measurements of relative electron transport rates (rETR) as a function of photon flux were carried out via Rapid Light Curves (RLCs; White and Critchley, 1999) using a 30 s exposure to each photon flux. The parameter F_v/F_m, was determined after a dark incubation of at least 10 min. This represents the maximum quantum efficiency of photosystem II and is often used to gauge plant health (~0.7–0.8 in healthy plants; Baker and Horton, 1987).

The RLCs were used to calculate the quantum efficiency of light harvesting (α), the photoinhibition coefficient (β), the level of light that saturates photosynthesis (Ik) and the maximum photosynthetic rate (ETR_{max}). ETR_{max} and α were calculated by fitting the calibrated P-I response curves of Sc to the exponential model of Webb et al. (1974). Photosynthesis-irradiance (P vs I) responses could not be determined, due to insufficient biomass (< 4 mg chlorophyll *a* m^{-2}) of MPB in Ly, Hu and most of the Do samples, so only RLCs from Sc were analyzed (median 9.55 mg Chl *a* m^{-2}).

Statistical analyses

The effects of stream, light and time on four dependent variables (Chl *a,* pheophytin, Chl *a:* pheophytin ratio and cell density) were assessed by a partly nested analysis of variance with one among-blocks factor (stream) and two crossed within-blocks factors (light and time). There were four levels of stream (corresponding to a gradient of urban stormwater impact, Table 1: Ly = 1, Hu = 2, Do = 3, Sc = 4), and six levels of time (T1 to T6). Three levels of light (L, M, H: or two levels, L and H, for recoded analysis; Fig. 4) were applied within each block of three plates per stream. All three factors were considered fixed. Probabilities for the within-blocks factors were adjusted for lack of sphericity using either the Greenhouse-Geisser ε statistic, if $\varepsilon < 0.75$, or the Huynh-Feldt statistic, if $\varepsilon \geq 0.75$ (Quinn and Keough, 2002). Where effects were significant ($P < 0.05$), the effect of increasing catchment urbanization, light, time or their interactions was tested by linear polynomial contrasts. Two blocks of replicates were missing from Do at time 2 (T2) due to field problems so T2 was excluded from the overall analysis.

The effect of stream, time and light on assemblage composition was interpreted using analysis of similarity (ANOSIM) and non-metric multidimensional scaling (MDS) in PRIMER (Clarke and Warrick, 1994), based on a Bray-Curtis similarity matrix using square-root-transformed relative abundance data. Taxon abundance was averaged for the three replicate composition samples in each stream. The individual effects of stream, light and time on assemblage composition were investigated separately in one-way ANOSIMs. Two-factor crossed ANOSIMs were also conducted to compare the effect of stream and light (pooled across times), and stream and time (pooled across light treatments).

Table 2. Results from overall ANOVAs that indicate the effect of stream, light and time, and their relative interactions, on Chl *a* concentration (original and recoded), pheophytin concentration, Chl *a* to pheophytin ratio and cell density. 'Recoded' denotes use of recoded light categories, all other analyses used original light categories. df, Degrees of freedom; MS, mean square; %V, percentage of variance, significant effects are indicated as * P<0.05, ** P<0.01 or *** P<0.001 where P, probability of a Type I error.

		Chl *a*		Pheophytin		Chl *a*:Pheophytin			Chl *a* (recoded)		Cell density	
Effect	df	MS	%V	MS	%V	MS	%V	df	MS	%V	MS	%V
Among blocks												
Stream	3	40.46***	58.9	5.97**	18.5	11.33**	26.0	3	25.39***	59.6	6195***	9.6
Error: Block(Stream)	7	1.02	3.5	0.44	3.2	0.89	4.8	7	0.31	1.7	227	0.8
Within blocks												
Light	2	0.57	0.6	0.01	0.0	0.08	0.1	1	1.65	1.3	753	0.8
Stream x Light	6	0.57	1.7	0.13	0.8	0.21	1.0	3	0.50	1.2	438	1.4
Error: Block(Stream) x Light	14	0.52	3.5	0.32	4.5	0.24	2.5	7	0.60	3.3	320	2.3
Time	4	4.66***	9.0	3.01***	12.4	0.52*	1.6	4	1.46**	4.6	5897*	3.0
Stream x Time	12	1.08*	6.3	0.42*	5.2	0.49**	4.5	12	0.82**	7.7	1454	2.2
Error: Block(Stream) x Time	28	0.51	6.9	0.17	5.0	0.16	3.5	28	0.27	5.8	969	3.5
Light x Time	8	0.37**	1.4	0.09	0.8	0.93	5.7	4	0.44	1.4	258	0.3
Stream x Light x Time	24	0.42***	4.9	0.17	4.3	0.36	6.7	12	0.43	4.1	822	2.5
Error: Residual	55	0.13	3.3	0.80	45.4	1.00	43.6	28	0.43	9.4	10188	73.6

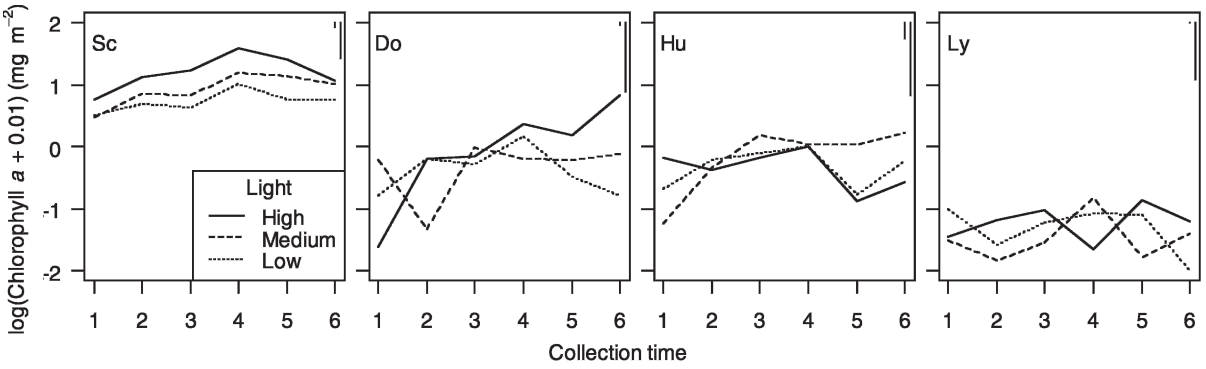

Figure 5. Mean algal biomass (Chl *a*) under each light treatment over time in each stream; minimum and maximum standard deviation bars in upper right corner of each plot.

Results

Algal biomass

Algal biomass, as indicated by Chl *a*, increased with greater catchment urbanization; it was linearly related to urbanization at all times and under all light treatments (all linear contrasts of the stream effect with increasing urbanization were significant at $P < 0.001$). Stream was the overriding factor that influenced Chl *a*, accounting for more than half of the variance in both original and recoded light categories (Table 2).

The relative effect of light and time on Chl *a* differed among sites (Fig. 5, Table 2). Chl *a* was greater under higher light treatments from T3 to T5 at Sc (significant linear contrasts for the light effect, Fig. 5). Although light did not seem to influence Chl *a* at Hu or Ly across different times, there was a significant linear light effect in Ly at T6. Interactions containing light only had a significant effect on Chl *a* when the three original light categories were used (Fig. 5, Table 2), suggesting the light effect in this analysis was driven by the observed increases for the high-light treatments (excluded from the recoded analysis) at the two most urban streams (Sc and Do).

The only significant increase in Chl *a* over time detected was for the medium-light treatment at Hu. The apparently strong increase in mean Chl *a* for the high-light treatment at Do (Fig. 5) was not significant.

Pheophytin increased with urbanization but it increased to a lesser extent and was explained less well by urbanization than Chl *a* (Fig. 6). Although pheophytin concentrations were influenced by stream, time and the stream-time interaction (Table 2), these differences were not explained by the hypothesized gradients; polynomial contrasts did not reveal a gradient effect of time or stream (original and recoded light categories). Cell density was positively correlated with stream urbanization and time but was not affected by light treatment (Table 2).

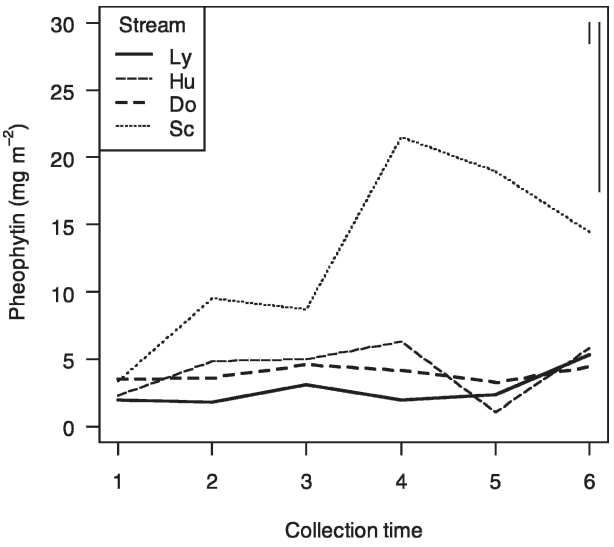

Figure 6. Mean pheophytin concentration (untransformed) in each stream relative to time (minimum and maximum standard deviation bars in upper right corner).

Assemblage composition

Stream was the only factor that had a significant effect on assemblage composition (Fig. 7, Table 3). The most obvious difference in assemblage composition among the streams was the greater relative abundance of filamentous chlorophytes in the most urbanized stream (Sc). Considering that the other streams were largely devoid of filamentous green algae (mean = 0.4 %), their 11 % contribution to the assemblage at Sc was high (Fig. 8). Consequently, Sc had the most distinct composition ($R > 0.8$ in all stream pairwise tests) (Table 4).

Table 3. Importance of stream, light treatment and time in influencing MPB assemblage composition as determined by Analysis of Similarities. Significant results indicated as *** P<0.001. All analyses used Bray-Curtis dissimilarity on square-root transformed data, 999 permutations.

Effect	Global R
One-way ANOSIMs	
Stream	0.577***
Light	–0.049
Time	–0.018
Two-way ANOSIM pooling times	
Light	–0.103
Stream	0.563***
Two-way ANOSIM pooling light treatments	
Time	0.103
Stream	0.670***

Table 4. The relative difference in assemblage composition at the four streams as determined by ANOSIM pairwise tests (significant results in bold, * P<0.05, ** P<0.01, *** P<0.001.) Data transformed – square root; Bray-Curtis dissimilarity; 999 permutations.

Streams		R Statistic
Do	**Hu**	**0.529*** **
Do	Ly	0.197*
Do	**Sc**	**0.815*** **
Hu	Ly	0.351**
Hu	**Sc**	**0.809*** **
Ly	**Sc**	**0.809*** **

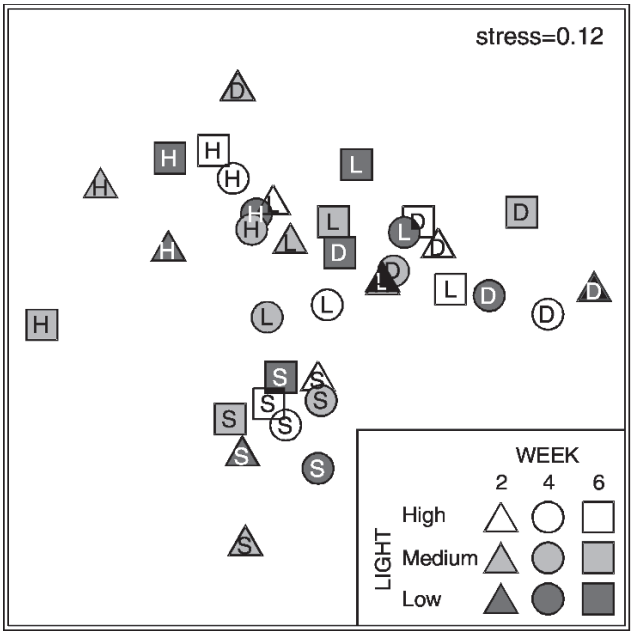

Figure 7. Multidimensional scaling plot of assemblage composition of MPB samples under the three light treatments on 3 sampling dates (weeks 2, 4 and 6) in each of the four streams, Sc, Do, Hu and Ly, indicated by their first letters.

All of the streams were dominated by pennate diatoms, which accounted for an average of 74 % of the cells counted (Fig. 8). Filamentous cyanobacteria were the second most abundant taxon (17 %), but this was not consistent throughout all of the streams (Fig. 8).

Physiological characteristics

MPB exposed to different light treatments differed physiologically (Fig. 9). The maximum rate of electron transport (ETR_{max}), which indicates photosynthetic rate, was consistently lowest, on an areal basis, in the MPB samples collected from low-light treatments, and was usually highest in the high-light samples. The P-I response curves, namely the rise in ETR_{max}, also demonstrated that photosynthetic capacity changed with time. In samples from high and medium-light treatments, ETR_{max} at T6 was ~3 x greater than at T1

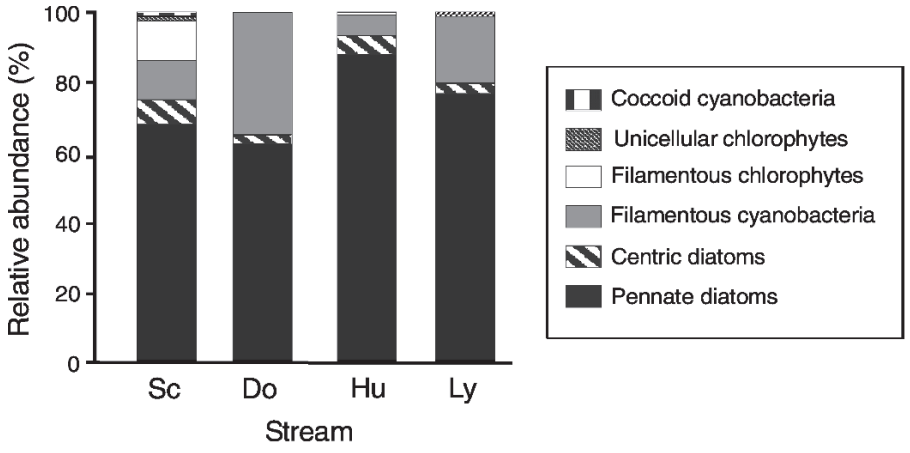

Figure 8. Mean relative abundance (pooled across dates) of major taxonomic groups in the four streams.

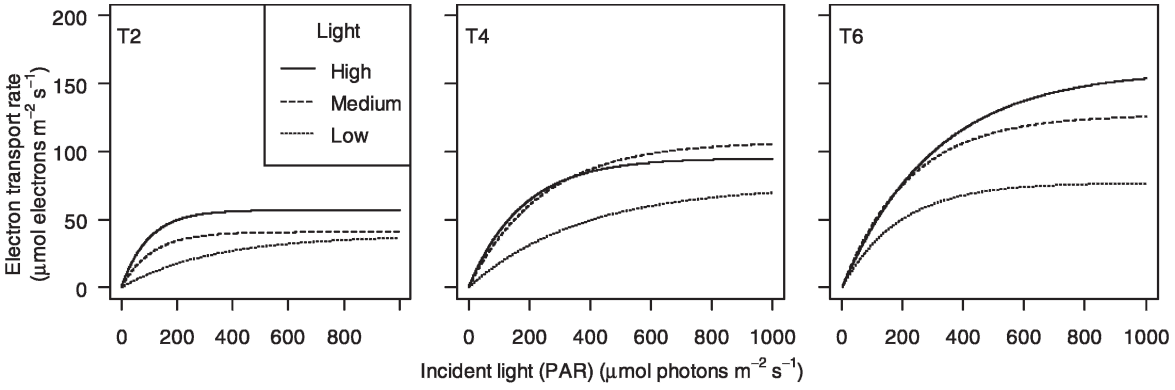

Figure 9. Mean photosynthesis-irradiance (P-I) responses of *Sc* MPB samples from high-, medium- and low-light treatments at times 2, 4 and 6. (Photosynthesis indicated by ETR, and irradiance by photosynthetically active radiation(PAR); Light: H – solid line, M – dashed line, L – fragmented line).

(~50 – 150 µmol electrons m-^{2} s-^{1}), reflecting the increase in population size.

The health of the MPB, as indicated by F_v/F_m, showed a positive correlation with time and light (Fig. 10a). Fv/Fm and α from each light treatment differed in the first half of the study, but tracked to a similar level in the second half (~0.7 and ~0.5 mol electrons µmol photons^{-1}, respectively; Fig. 10b). The rate at which Fv/Fm and α stabilized was positively correlated with light. The light level required to saturate photosynthesis (Ik) decreased with time and became stable at ~200 µmol photons.

Discussion

Differences among streams (linearly related to the urban gradient represented by our four study sites) explained a large proportion of variance in MPB biomass and composition in the four study streams under controlled light conditions. While light intensity was low in this winter-time study, the response of MPB biomass (as indicated by Chl *a*) to light differed among the four streams; there was no response in the two least urban sites (Ly and Hu), a delayed response to the highest light treatment at Do, and a consistent response to all three light treatments at the most urbanized site (Sc).

We postulate that the positive correlation between MPB biomass and urbanization observed by Taylor et al. (2004) in small streams to the east of Melbourne was driven by factors other than light (Fig. 1). As discussed below, the most likely urban-associated impact responsible for the observed pattern is an increase in available nutrients (Fig. 1; Taylor et al., 2004) (Hillebrand, 2002).

Despite the positive response to light in the two most urbanized streams, the MPB in all of the streams were potentially light limited. Our analysis of photophysiological characteristics of the MPB, as well as studies by Roberts et al. (2004), Richardson et al. (1983) and Wellnitz and Ward (1998) (river periphyton limited at 286 µmol photons m^{-2} s^{-1}), indicate that even the high-light treatment at Sc (43.1µmol photons

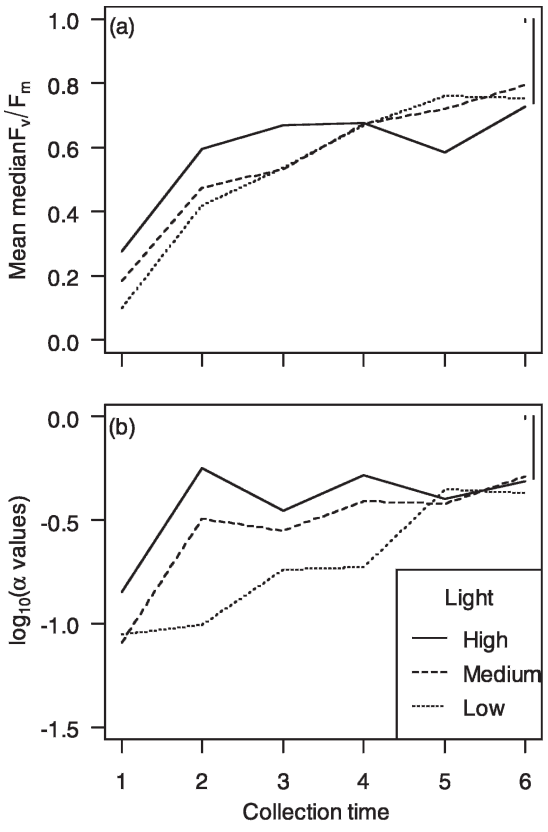

Figure 10. (a) Mean Fv/Fm values (each value the median of 6 trials) of MPB samples from the three light treatments at *Sc* over time (with standard deviation bars). (b) Mean *a* values of MPB samples from the three light treatments at *Sc* over time (with standard deviation bars).

$\bar{m}^2\ \bar{s}^1$) would have limited MPB growth. F_v/F_m and α from the different light treatments tracked to the same level over time indicating photoacclimation as assemblages on the slides developed (Fig. 10; Falkowski and LaRoche, 1991; Atwell et al., 1999); the rate at which they reached the optimum physiological state was a function of available energy (light). The increasing α with time suggests the onset of light acclimation as assemblages caused self shading. ETR_{max} values level off to different values depending on light treatment (Fig. 9), which reflects the changing population density on the slides but is also typical of light acclimation responses of microalgae (Richardson et al., 1983).

The dominance of diatoms and cyanobacteria in MPB assemblages reflects the low-light conditions (Fig. 8). Pooling data from numerous studies, Richardson et al. (1983) found that Cyanophyceae (cyanobacteria) and Bacillariophyceae (diatoms) had a mean optimum photon flux of 38.8 μmol photons $\bar{m}^2\ \bar{s}^1$ and 84.0 μmol photons $\bar{m}^2\ \bar{s}^1$ respectively compared to 211 μmol photons $\bar{m}^2\ \bar{s}^1$ for Chlorophyta (chlorophytes).

Although not optimum, the photon fluxes at Hu and Ly were above the light compensation points of diatoms and cyanobacteria (except the low-light treatment) (Richardson et al., 1983) and MPB were physiologically healthy as indicated by F_v/F_m values (Kobe et al., 1995) so population growth was viable. MPB in these streams appeared to be limited by nutrient levels rather than light, consistent with a study of stream periphytic diatoms in British Columbia (Bothwell, 1988).

Whereas Ly and Hu MPB were primarily nutrient limited and Sc light limited, the growth of only the high-light MPB at Do suggests that Do algae suffer from both nutrient and light limitation. MPB at Do were able to cope with nutrient limitation, contesting Rosemond's (1994) hypothesis that periphyton growth can only occur in co-limited systems if both factors are elevated, with sufficient light (H: >32.2 μmol photons $m^{-2}s^{-1}$) and time (T4: 7 weeks). Consequently, increases in either phosphorus or irradiance should stimulate algal population growth in Do, as was seen in phytoplankton of the Colne Estuary in the UK (Kocum et al., 2002).

Urbanization-induced changes to flow characteristics and herbivory are unlikely to have caused the observed biomass-urbanization pattern. Flow disturbance increases with urbanization (Fig. 1) so disturbance events would be more frequent and of a greater magnitude in streams of more urbanized catchments (Taylor et al., 2004). For example, it is likely that higher flows following a 15 mm rain event (Australian Bureau of Meteorology records) caused the decline in peak biomass at T4 in Sc (J. Catford, personal observation) as cells became more susceptible to sloughing as growth exceeded the boundary layer of the slides (Hudon et al., 1987; Biggs, 1995). Consequently, the observed pattern of increasing MPB biomass with urbanization would have been conservative; disturbance can reduce algal biomass (e.g. Biggs, 1995; Biggs et al., 1998) and can override the influence of nutrient enrichment (Biggs and Thomsen, 1995).

Similarly, if grazing pressure varied among streams (though qualitative observations did not suggest any differences: J. Catford, personal observation) we would expect it to increase from Ly to Sc; after a time-lag, herbivore density increases in response to algal population growth (Rosemond et al., 1993; Bergey, 1995; Anderson et al., 1999; Hillebrand, 2002). However, a temporal change in assemblage composition was not detected (herbivores usually graze selectively; Anderson et al., 1998) and the proportion of live and decomposing algae was stable. This suggests that grazing was not important in this study.

Although there was an increase in α with time, indicating acclimation to lower irradiance, due to the relatively low biomass of the slides compared with studies that have reported self-shading (This study: 0–73 mg chl. *a* m^{-2}; Boston and Hill (1991): 30–860 mg chl. *a* m^{-2}; see also Hudon et al. (1987); Hill (1996)), it was assumed that any effect from self-shading would have been negligible compared to the effect of light treatment.

In conclusion, with light controlled, large differences in MPB biomass in small streams of eastern Melbourne were explained by differences in catchment urbanization. We hypothesize that this effect is primarily driven by increased phosphorus concentrations in more urbanized streams delivered by diffuse stormwater runoff. When nutrient limitation is alleviated, secondary influences take effect. These influences include light, and possibly physical disturbance and grazing. Not all of the limitations need be alleviated before an increase in biomass is observed – with enough light at Do, the impact from nutrient limitation was reduced.

While studies on urban streams in the eastern USA (Hession et al., 2004; Roy et al., 2005) have found that open reaches have significantly greater algal biomass than reaches with forested riparian cover, algal biomass in streams of our study was more strongly influenced by catchment urbanization than by shading. To our knowledge, our study is the first that experimentally separates the effect of light from other factors influenced by urbanization. Our results suggest that nutrient levels must be managed at the catchment-scale to prevent a large increase in algal biomass in, at least, some urban streams. This could be achieved by reducing connection of stormwater drainage for example (Hatt et al., 2004; Taylor et al., 2004). A more pragmatic approach in the short-term would be provision of shade with riparian vegetation and prevention of channel widening. However, even at very low-light levels, streams with highly urbanized catchments may support an algal biomass two orders of magnitude greater than nutrient-limited streams (Fig. 5).

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