A pigment composition analysis reveals community changes in pre-established stream periphyton under low-level artificial light at night

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A pigment composition analysis reveals community changes in pre-established stream periphyton under low-level artificial light at night

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Abstract

Freshwaters are increasingly exposed to artificial light at night (ALAN), yet the consequences for aquatic primary producers remain largely unknown. We used stream-side flumes to expose three-week-old periphyton to LED light. Pigment composition was used to infer community changes in LED-lit and control periphyton before and after three weeks of treatment. The proportion of diatoms/chrysophytes decreased (14%) and cyanobacteria increased (17%) in lit periphyton in spring. This may reduce periphyton nutritional quality in artificially-lit waters.

Keywords: ALAN; algae; biofilm; HPLC; LED; light pollution

Due to urbanization and the spread of electrical lighting, freshwaters are increasingly exposed to artificial light at night (ALAN) (Hölker et al., 2010; Falchi et al., 2016). Several studies have reported the ecological effects of ALAN, but its impacts on freshwaters, particularly aquatic primary producers, remain understudied (Perkin et al., 2011). Light is a key resource for autotrophs and regulates numerous physiological processes through circadian clocks (Hegemann et al., 2001). Autotrophs within periphyton communities form the base of aquatic food webs in clear, shallow waters including streams (Stevenson, 1996; Law, 2011). A recent study in a stream-side flume system (Grubisic et al., 2017), using *in-situ* fluorometer (BenthoTorch), found that three weeks of exposure to ALAN decreased periphyton biomass and the proportion of cyanobacteria, and increased the proportion of diatoms in periphyton in the early growth stages (up to three weeks). No effects were detected in later growth stages (three to six weeks). Here, we applied the more conventional method, high-performance liquid chromatography (HPLC) on the same periphyton communities in later growth stages and used pigment composition to assess

community composition. HPLC separates photosynthetic pigments in mixed algal assemblages (Millie et al., 1993). This method might reveal ALAN-induced changes in community composition in the later periphyton stages that were not detected with the fluorometer.

The flume system used in this study and details of the experimental design are described in Grubisic et al. (2017). Briefly, five U-shaped flumes (20 m long, 30 cm wide, with 30 or 50 cm high side walls) were fed with water from the adjacent Fersina stream (Trentino, Italy, 46° 04′ 32″ N, 11° 16′ 24″ E). Sixteen unglazed ceramic tiles (9.8 cm x 19.6 cm) were placed on top of a cobble layer across the length of the flumes and left for 26 days in spring and 22 days in autumn to facilitate development of a "pre-established" periphyton community (Oemke and Burton, 1986 and references therein). Longer periods were avoided to prevent periphyton entering the senescence phase that could drive biomass independently of ALAN. The studied periphyton was thus past the initial colonization phase but still developing during the experiment. Artificial lights (LED strips, 3000 K, 20.3 ± 1.8 lux at the water surface, mean and SD) were installed above either the upper or lower section (randomized) of each flume, resulting in five lit and five control sections. During the following three weeks of experimental treatment, lights were turned on from civil twilight until morning. We applied a replicated BACI (Before-After-Control-Impact) approach: four tiles per flume section were sampled before lights were switched on (March 31 and September 24) and at three weeks of experimental treatment (April 23 and October 16). Environmental parameters (oxygen, temperature, pH, conductivity, velocity, turbidity) and initial densities of macroinvertebrates were similar between the treatments, thus not confounding with the effects of ALAN, as described in Grubisic et al. (2017).

Tiles were carefully removed from the flumes and non-periphytic material (e.g., Simuliidae larvae) was removed with forceps. Periphyton was brushed from the tiles and two

aliquots were concentrated on Whatman GF/F filters, for determination of dry mass and pigment composition. Filters for dry mass were dried to the constant weight at 60°C and weighed. Filters for pigment analysis were transferred to -80°C for 48 hours to stimulate cell lysis and subsequently freeze-dried and stored at -20°C. Pigments were analyzed following the procedure described in Woitke et al. (1994). Pigments were identified and quantified by their retention time and absorption spectra compared with standards and values from the literature (Jeffrey et al., 1997). Chlorophyll *a* (Chl *a*) was calculated as the sum of the true chlorophyll *a* and chlorophyllids *a* and determined as a mean of the absorption readings at 440 and 410 nm wavelength. All other pigments were determined from the absorption readings at 440 nm.

Pigment concentrations were normalized to the Chl *a* concentration, z-standardized and subjected to principal component analysis (PCA) using packages vegan (Oksanen et al., 2015) and shape (Soetaert, 2014) in R (R Core Team, 2015). PCAs were computed separately for each season. The values were log-transformed for autumn data to meet the assumption of normal distribution. Scores of PCA axes were tested using linear mixed-effects models (LMM) (Zuur et al., 2009) including treatment (lit and control) and time (before and after) as fixed factors, and flume as a random factor. A significant interaction treatment x time indicates an effect of ALAN on periphyton community composition. Pairwise comparisons of significant interactions were performed using the multcomp package (Hothorn et al., 2008) with Benjamini-Hochberg *p* correction.

Eight pigments were identified in our samples (Table 1). Chl *a* was the most abundant pigment in both lit and control periphyton, with values comparable to those obtained by *in situ* fluorometry on the same communities (Grubisic et al., 2017). Chl *a* increased with time in both seasons (time effect in spring: $F_{(1,70)} = 179.9$, $p < 0.001$; in autumn $F_{(1,70)} = 318.4$, $p < 0.001$), but

its concentrations did not differ between lit and control periphyton in either season (treatment x time in spring $F_{(1,70)} = 0.30$, $p = 0.57$; in autumn $F_{(1,70)} = 0.03$, $p = 0.86$).

	Before treatment		After treatment	
Pigment	Control (D)	Lit (L)	Control (D)	Lit (L)
Spring				
Chlorophyll a	0.136 ± 0.063	0.146 ± 0.087	1.322 ± 0.914	1.247 ± 1.025
Chlorophyll b	n.d.	n.d.	0.046 ± 0.056	0.104 ± 0.148
Chlorophyll c	0.013 ± 0.006	0.015 ± 0.009	0.125 ± 0.104	0.102 ± 0.103
Fucoxanthin	0.029 ± 0.014	0.032 ± 0.02	0.349 ± 0.277	0.319 ± 0.301
Violaxanthin	0.005 ± 0.002	0.006 ± 0.004	0.054 ± 0.041	0.040 ± 0.032
Diadinoxanthin	n.d.	n.d.	0.022 ± 0.017	0.022 ± 0.017
Diatoxanthin	n.d.	n.d.	n.d.	n.d.
Zeaxanthin	n.d.	n.d.	0.011 ± 0.009	0.010 ± 0.010
Autumn				
Chlorophyll a	0.052 ± 0.024	0.045 ± 0.02	0.252 ± 0.125	0.326 ± 0.175
Chlorophyll b	n.d.	n.d.	n.d.	n.d.
Chlorophyll c	0.004 ± 0.002	0.003 ± 0.002	0.018 ± 0.010	0.024 ± 0.015
Fucoxanthin	0.013 ± 0.008	0.012 ± 0.006	0.071 ± 0.041	0.092 ± 0.057
Violaxanthin	n.d.	n.d.	n.d.	n.d.
Diadinoxanthin	0.002 ± 0.001	0.002 ± 0.001	0.009 ± 0.003	0.011 ± 0.008
Diatoxanthin	0.002 ± 0.002	0.001 ± 0.001	0.004 ± 0.003	0.004 ± 0.002
Zeaxanthin	0.001 ± 0.001	n.d.	0.005 ± 0.003	0.006 ± 0.003

Table 1. Pigment concentrations (µg cm^{-2}) measured by high-performance liquid chromatography (HPLC) in periphyton in the two seasons (mean ± SD, $n = 160$), before and after experimental treatment, in the control (D) and lit (L) flume sections.

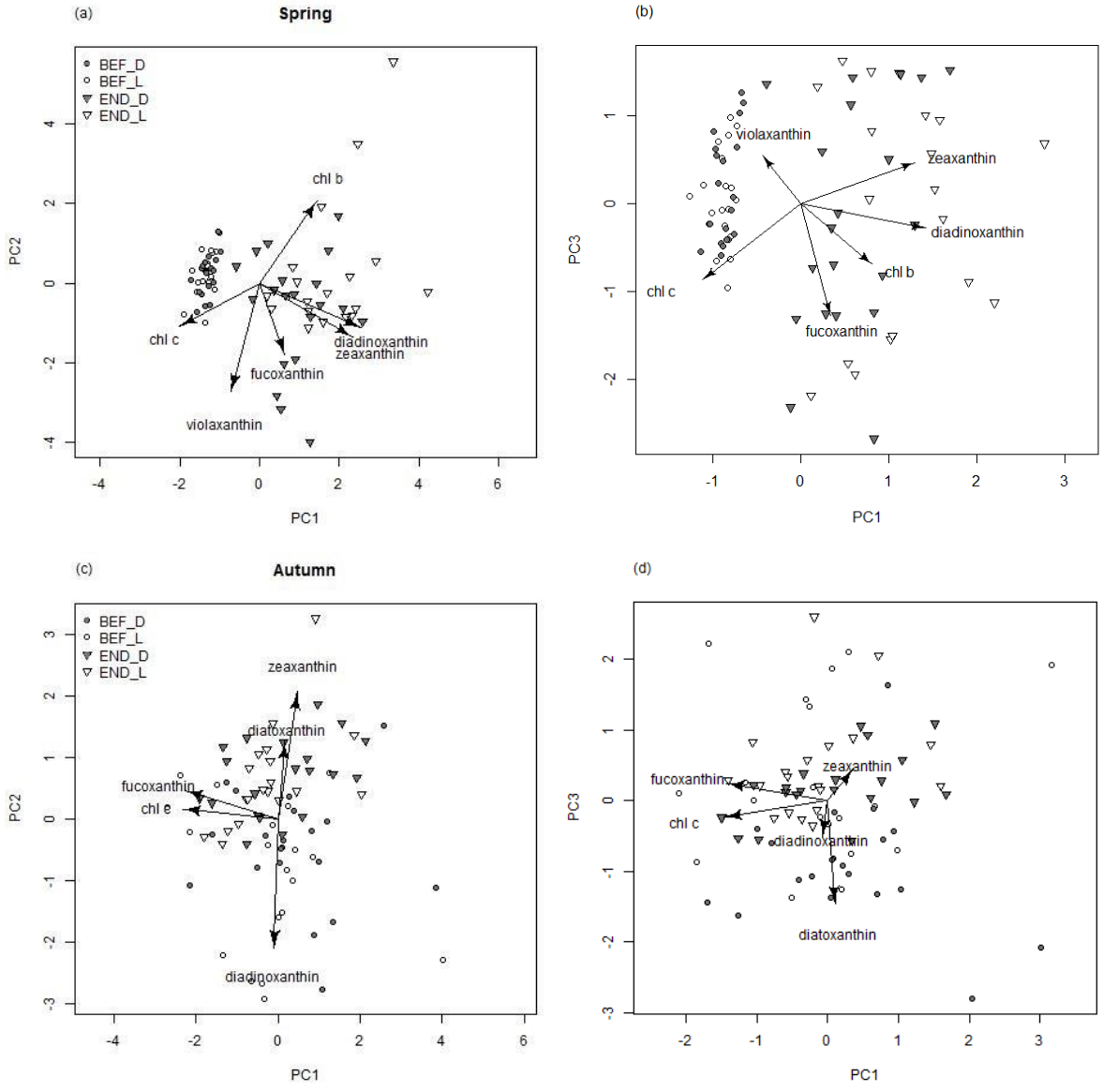

Figure 1. Correlation biplots produced by principal component analysis (PCA) based on relative pigment concentrations of periphyton in the two seasons (n = 80); before ("BEF") and after ("AFT") experimental treatment, in the control (D) and lit (L) flume sections. Plane of axes one (PC1) and two (PC2) (a, c) and one and three (PC3) (b, d) are shown. Amount of variation explained by the planes: (a) 64%, (b) 61%, (c) 59%, (d) 53%. Arrows show correlation between original pigment variables with PC axes and all indicate significant relationship ($p < 0.0001$).

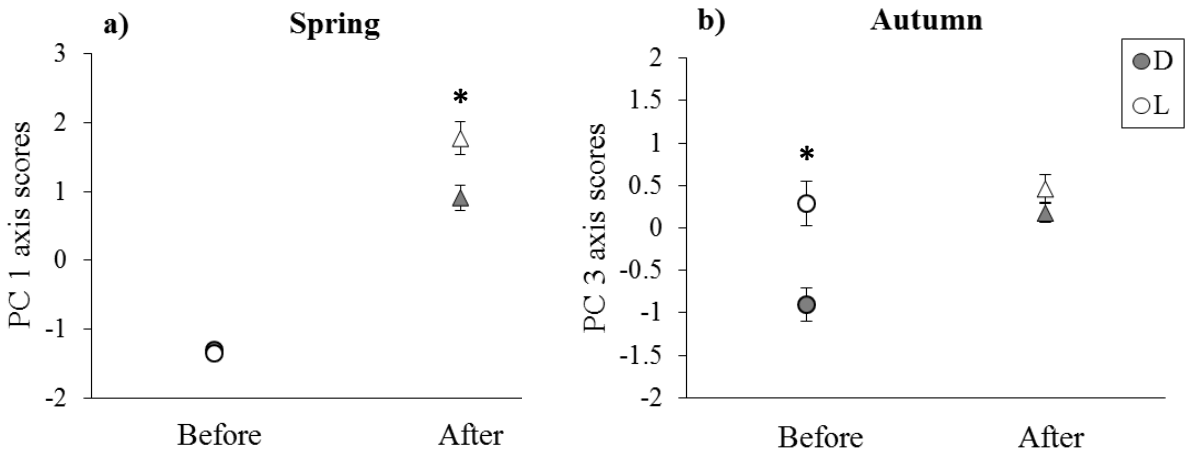

Figure 2. Scores of principal component axes that are significantly affected by the artificial light at night in the two seasons (mean ± SE); before and after the experimental treatment, in control (D) and lit (L) periphyton. Amount of variation explained by the PC axes: (a) 39%, (b) 20%. Asterisks indicate significant differences (LME, pairwise comparisons with Benjamini-Hochberg correction, in spring $p = 0.005$, in autumn $p = 0.004$).

In spring, 86% of the total variance could be explained by principal components (PC) with axes 1 (39%), 2 (25%) and 3 (22%). Time and treatment-induced variation in pigment composition were visible along PC1 and PC2 (Fig.1a). PC1 correlated with diadinoxanthin (Pearson's $r = 0.90$), zeaxanthin ($r = 0.82$) and Chl *c* ($r = -0.70$) and its scores were significantly affected by ALAN (Fig. 2a; treatment x time $F_{(1,70)} = 8.76$, $p = 0.004$). Pairwise comparisons identified no differences in PC1 scores between lit and control periphyton before the treatment ($p = 0.42$) and higher scores in lit periphyton after ALAN treatment ($p = 0.005$) compared to the control. This indicated that relative concentrations of diadinoxanthin and zeaxanthin increased in lit periphyton relative to the control, while Chl *c* decreased (Table 1). PC2 correlated with violaxanthin (Pearson's $r = -0.76$) and Chl *b* ($r = 0.58$) and PC 3 with fucoxanthin ($r = -0.8$) and

with Chl *c* (r = -0.54). Scores of PC2 and PC3 were not affected by ALAN (treatment x time for PC2 $F_{(1,70)} = 3.44$, $p = 0.07$; for PC3 $F_{(1,70)} = 0.24$, $p = 0.62$).

In autumn, the first three principal components explained 78% of the total variance (33%, 26% and 20%, respectively). Time-induced variation in pigment composition was mainly visible along PC1 (Fig.1c), that correlated with Chl *c* (Pearson's r = -0.89) and fucoxanthin (r = -0.91). PC2 correlated with diadinoxanthin (r = -0.78) and zeaxanthin (r = -0.80). Scores of PC1 and PC2 were not significantly affected by ALAN (treatment x time for PC1: $F_{(1,70)} = 0.74$, $p = 0.39$; for PC2: $F_{(1,70)} = 1.27$, $p = 0.26$). Scores of PC3 were correlated with diatoxanthin (r = -0.79) and significantly affected by ALAN (Fig. 2b; treatment x time $F_{(1,70)} = 4.64$, $p = 0.03$). Pairwise comparisons identified significant differences in scores between the lit and the control periphyton prior ($p = 0.004$), but not after the treatment ($p = 0.24$). Before the treatment, lit periphyton had significantly lower relative concentrations of diatoxanthin than control periphyton (Table 1), but concentrations were similar after exposure to ALAN. The difference between lit and control sections before the treatment was likely stochastic variation, thus the convergence of pigment composition in autumn does not necessarily reflect an effect of ALAN.

Ratios of photosynthetically active pigments (Chl *a*, Chl *c* and fucoxanthin) to periphyton dry mass were not significantly affected by ALAN in either season. Chl *b* was excluded from this analysis, as it was present only rarely.

Chl *a* is a common estimator of autotroph biomass, as it is found in all photosynthetic organisms (Stevenson, 1996). An increase in Chl *a* with time in both lit and control periphyton indicated that biomass of autotrophs increased throughout the experiment, but no effects of ALAN on biomass were found. In a previous study, *in situ* fluorometry identified diatoms as the dominant group in pre-established periphyton in both seasons and their proportion in the

community was not affected by ALAN (Grubisic et al., 2017). Here, however, using a more sensitive method, HPLC pigment analysis, we were able to identify ALAN-induced changes in periphyton composition. In spring, Chl *c* was 14% lower in lit periphyton compared to the control. Chl *c* is characteristic for algal groups of the red lineage (Jeffrey et al., 1997), in freshwater periphyton mainly diatoms and chrysophytes (Stevenson, 1996). Fucoxanthin is also associated with red lineage algae, but was not affected by ALAN. Both diadinoxanthin (nonspecific) and zeaxanthin (characteristic for green algae and cyanobacteria) (Jeffrey et al., 1997) had higher relative concentrations in lit periphyton then in the control (12% and 17%, respectively). Because Chl *b*, characteristic for green algae, was rarely detected, we conclude that increased zeaxanthin suggested an increase in cyanobacteria. For the autumn periphyton, the observed changes in diatoxanthin, another non-specific pigment (Jeffrey et al., 1997) can only be interpreted as an overall community change. The impacts of ALAN were significant in spring (14% decrease in Chl *c*, 12% increase in diadinoxanthin and 17% increase in zeaxanthin in lit periphyton compared to the control) but less clear in autumn (57% higher diatoxanthin in lit periphyton before, no differences between lit and control periphyton after the ALAN treatment). This seasonal variation was likely associated with seasonal differences in environmental characteristics (Grubisic et al., 2017) and in species composition (unpublished data). Because spring communities tend to be adapted to low-light conditions of the preceding winter (Laviale et al., 2009), periphyton in spring may be more sensitive to low-light levels such as those supplied by ALAN.

Algae can maximize photosynthetic efficiency under low light levels by increasing their cell pigment content (Falkowski and Raven, 2007). ALAN did not affect ratios of photosynthetic pigments to dry mass, indicating that periphyton did not physiologically acclimate to ALAN. The

constant ratio of Chl *a* to dry mass also indicated that ALAN did not increase the proportion of autotrophs in the periphyton.

Algal food quality is an important regulator of trophic efficiency of energy flow through stream ecosystems (Guo et al., 2016) and by altering the nutritional quality of primary producers, ALAN may affect food supply for higher trophic levels and food web dynamics in stream ecosystems. Periphytic algae are an important food source in streams. By decreasing the proportion of red lineage algae (14%) and increasing the proportion of cyanobacteria (17%), ALAN may decrease the nutritional quality of periphyton in later growth stages (three to six weeks). Diatoms are a preferred food source for grazers because of their high omega-3 polyunsaturated fatty acids content, in contrast to cyanobacteria which are considered to be a low-quality food source (see Guo et al., 2016). In early growth stages (one to three weeks) the opposite effects of ALAN were observed, with decrease (11%) in the proportion of diatoms at two weeks but an increase (5%) at three weeks in autumn, and a decrease in the proportion of cyanobacteria (54% at three weeks) in spring.

Periphyton plays a key role in primary production, nutrient cycling and food web dynamics in many clear, shallow waters, thus ALAN may alter species dominance patterns and have consequences for higher trophic levels through changes in food quality. It was previously reported that stream periphyton in early growth stages (one to three weeks) is sensitive to ALAN (Grubisic et al., 2017) and here we found it is also true for later growth stages (three to six weeks). Detection of impacts of ALAN on periphyton might therefore require application of sensitive analytical methods such as HPLC or taxonomical identification. By changing the proportions of diatoms and cyanobacteria in the periphyton, two groups of primary producers

with contrasting nutritional quality, ALAN may affect the food quality for primary consumers. The consequences for food web dynamics and secondary production in streams remain unknown.

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