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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.

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 lx 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.

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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 h 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 Development 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 $F_{(1,70)} = 0.03$, $p = 0.86$).

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

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.

	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