A transition to white LED increases ecological impacts of nocturnal illumination on aquatic primary producers in a lowland agricultural drainage ditch

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A transition to white LED increases ecological impacts of nocturnal illumination on aquatic primary producers in a lowland agricultural drainage ditch

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Abstract

The increasing use of artificial light at night (ALAN) has led to exposure of freshwater ecosystems to light pollution worldwide. Simultaneously, the spectral composition of nocturnal illumination is changing, following the current shift in outdoor lighting technologies from traditional light sources to light emitting diodes (LED). LEDs emit broad-spectrum white light, with a significant amount of photosynthetically active radiation, and typically a high content of blue light that regulates circadian rhythms in many organisms. While effects of the shift to LED have been investigated in nocturnal animals, its impact on primary producers is unknown. We performed three field experiments in a lowland agricultural drainage ditch to assess the impacts of a transition from high-pressure sodium (HPS) to white LED illumination (color temperature 4000K) on primary producers in periphyton. In all experiments, we compared biomass and pigment composition of periphyton grown under a natural light regime to that of periphyton exposed to nocturnal HPS or, consecutively, LED light of intensities commonly found in urban waters (approximately 20 lux). Periphyton was collected in time series (1 - 13 weeks). We found no effect of HPS light on periphyton biomass; however, following a shift to LED the biomass decreased up to 62%. Neither light source had a substantial effect on pigment composition. The contrasting effects of the two light sources on biomass may be explained by differences in their spectral composition, and in particular the blue content. Our results suggest that spectral composition of the light source plays a role in determining the impacts of ALAN on periphyton and that the ongoing transition to LED may increase the ecological impacts of artificial lighting on aquatic primary producers. Reduced biomass in the base of the food web can impact ecosystem functions such as productivity and food supply for higher trophic levels in nocturnally-lit ecosystems.

Keywords: artificial light at night; biofilm; light pollution; periphyton; urban stressor

Capsule: A switch from nocturnal high-pressure sodium to white LED illumination decreased the biomass of periphyton in a field study; therefore a transition to LEDs in outdoor lighting may increase ecological effects of light pollution in illuminated waters.

Introduction

The use of artificial light at night (ALAN) has rapidly increased in recent decades, leading to light pollution – an increase of nocturnal light above natural levels, and a disruption of the natural light/dark regime in many areas worldwide, with negative impacts on the environment. Almost 25% of the world's nonpolar land surface experiences light pollution (Falchi *et al.*, 2016). This widespread environmental alteration contributes to global change (Kyba *et al.*, 2017a), and raises concerns about the potentially adverse effects on organisms and processes in illuminated ecosystems (Hölker *et al.*, 2010a, Rich & Longcore, 2006). Along with an increase in nocturnal light levels, a global shift in outdoor lighting technologies from narrow-spectrum, e.g. yellow high pressure sodium (HPS), to broad-spectrum, white light-emitting diode (LED) lamps is taking place, driving a spectral shift in the nightscape (Kyba *et al.*, 2017a). Replacement of traditional lighting technologies by energy efficient LEDs is being implemented worldwide to decrease CO_2 emissions, environmental impacts, energy consumption and lighting costs (Hölker *et al.* 2010b). However, there are concerns about ecological impacts of LEDs (Davies *et al.*, 2017, Pawson & Bader, 2014, Ouyang *et al.*, 2017, Stone *et al.*, 2012) and the potentially negative consequences for human health (American Medical Association, 2016) associated with blue light in nocturnal illumination, which is found in commonly used white LEDs. The shift to LED lighting is expected to increase the ecological impacts of ALAN for many organisms (Gaston *et al* 2012), but this hypothesis has only been experimentally tested for terrestrial animals (e.g. bats, Lewanzik *et al.* 2017, Rowse *et al.* 2016).

A substantial fraction of freshwater ecosystems is increasingly exposed to light pollution worldwide. Estimates are that 80% of the human population lives in light-polluted areas (Falchi *et al.*, 2016), more than half of the total population being situated within 3 km of a body of freshwater (Kummu *et al.* 2011). With increasing population densities settling closer to freshwaters (Ceola *et al.* 2015), these ecosystems experience drastic environmental changes, including changes in light regime due to ALAN. Impacts of light pollution on freshwaters, as on aquatic ecosystems in general are, however, substantially less studied compared to terrestrial ecosystems (Perkin *et al.* 2011, Gaston *et al.* 2014). Furthermore, the majority of studies on freshwaters have focused on invertebrates (e.g. Manfrin *et al*., 2017, Moore *et al.* 2008, Perkin *et al.*, 2014a, 2014b, Thomas *et al.* 2016) and fish (e.g. Brüning *et al.*, 2015, 2018, Foster *et al.* 2016, Newman *et al.* 2015, Riley *et al.*, 2012, 2015), while impacts of ALAN on microorganisms and primary producers, are still scarcely studied (e.g. Hölker *et al.* 2015, Grubisic *et al.* 2017).

Primary producers use light as a source of energy for photosynthesis and as a source of information about the external environment (Hegemann *et al.*, 2001). Intensity, spectral composition, timing, and duration of light all influence photosynthesis (Falkowski & LaRoche, 1991, Fortunato *et al.*, 2015), and ALAN may stimulate photosynthesis at times when it would not naturally occur (Aube *et al.*, 2013). To contribute to net photosynthesis, however, ALAN has to provide enough energy to activate the photosynthetic machinery and to reach the photosynthetic compensation point. The intensity of illumination provided by outdoor lighting (typically < 0.002 % of daytime light levels) is often considered to be too low to stimulate photosynthetic production, except in directly illuminated areas (Gaston *et al.*, 2013). Theoretically, photosynthesis can occur at light levels slightly higher than moonlight, i.e. 0.1 µmol m^{-2} s^{-1}, approx. 0.5 – 7.4 lux (compared to the maximum moon light of 0.005 µmol m^{-2} s^{-1}, or approx. 0.3 lux, Kyba *et al.* 2017b) (Raven & Cockell, 2006), but the minimum thresholds in a real-world setting are not well known. The light utilized in

photosynthesis, photosynthetically active radiation (PAR), ranges from 400 to 700 nm, but blue (450 - 475 nm) and red light (630 - 675 nm) are utilized most efficiently. A light source with strong emission in these spectral regions is thus more likely to stimulate photosynthesis (Aube *et al.*, 2013). Furthermore, as light also provides information about the external environment, changes in light intensity and quality are detected by photoreceptors and conveyed for entrainment of the circadian clock that synchronizes internal physiological processes with the external light/dark cycle (Fortunato *et al.*, 2015). In particular, blue light perceived by cryptochromes and other flavin-containing is responsible for entrainment of the circadian clock, which is crucial for photosynthesis, growth, and survival of algae (Fortunato *et al.*, 2015, Dodd *et al.*, 2005). Disruption of natural light/dark cycles by ALAN, especially by light sources with high blue content, may disrupt circadian regulation. White LED light, with PAR efficiency at least twice than that of HPS (80-100% compared to maximum 40% of HPS) (Darko *et al.*, 2014), and typically a high content of blue light in the emission spectra, is therefore expected to have stronger impacts on aquatic primary producers compared to yellow HPS light.

A few field and laboratory studies have investigated effects of ALAN on aquatic primary producers, but the comparison of effects reported in these studies is confounded by different light sources, light intensities, and studied experimental systems. Poulin *et al.* (2014) found that short-term exposure to low-intensity HPS light (6.6 lux, approx. 0.08 µmol m^{-2} s^{-1}) affected several photophysiological processes in cyanobacterial cultures *(Microcystis aeruginosa)*, decreasing photosynthetic efficiency but not affecting growth. In microbial sediment communities, one year of similar light exposure in the field (HPS light, 6.8 – 8.5 lux, approx. 0.09 µmol m^{-2} s^{-1}) was found to alter community composition, increasing the relative abundance of diatoms and cyanobacteria (Hölker *et al.* 2015). When the same sediments were incubated in the laboratory under higher intensity white LED light (71 lux, approx. 1.3 µmol m^{-2} s^{-1}, color temperature 6300 K), nocturnal photosynthesis was stimulated

(Hölker *et al.*, 2015). In contrast, nocturnal illumination by white LEDs of intermediate intensity (20 lux, approx. 0.31 µmol m^{-2} s^{-1}, 3000K) decreased the biomass of periphyton in a sub-alpine stream in a field study, and altered the proportions of diatoms and cyanobacteria with the contrasting patterns (Grubisic *et al.* 2017, 2018). The role of spectral composition of ALAN in its impact remains unclear, therefore whether a spectral shift in the nightscape can affect aquatic primary producers is unknown. Whether ALAN affects only light-naïve communities, or also those from areas that have previously experienced light pollution, is not known.

We performed three experiments in a shallow, lowland agricultural ditch to assess the effects of a shift from traditional HPS to white LED lighting, on periphyton. To mimic the shift in outdoor lighting technologies, the HPS experiments were performed in 2014 (in summer and winter), and the LED experiment was conducted in winter 2015. Periphyton was collected in time series (1 - 13 weeks). In each experiment, the biomass and pigment composition of periphyton exposed to nocturnal illumination were compared with those of periphyton grown under a natural light regime. We expected yellow light from HPS lamps to have a weak impact on periphyton, due to the mismatch of its spectral composition with the sensitivity of photosynthetic pigments and its low blue content. We expected white LED light to have a strong impact on primary producers, by stimulating photosynthesis through a greater supply of PAR, and/or by disrupting circadian regulation because of high blue content. Therefore, a shift from HPS to LED would increase impacts of ALAN on periphyton. As periphyton is formed by a mixture of species that differ in their sensitivity to light and photosynthetic optima (Jeffrey *et al.*, 1997), we expected ALAN to differently affect individual taxa. This would drive a change in periphyton community composition that would be reflected in an altered pigment composition.

Material and methods

Sampling sites

The experiments were conducted in 2014 and 2015 in a lowland freshwater system within the rural area of Westhavelland Nature Park, located ca. 70 km northwest of Berlin, in Brandenburg, Germany. The park has little artificial nocturnal illumination and has been classified as a "Dark-Sky Reserve" by the International Dark-Sky Association (IDA). Three experimental sites (Holzhauer *et al.* 2015) were chosen along an approx. 5m wide agricultural drainage ditch (Fig. 1). The ditch is characterized by low flow velocity and soft sediment with a mean annual depth of 50 cm. The treatment (Lit) site contained commercial streetlights, and was designed to mimic street lighting conditions of urban areas. Illumination started in summer 2012 and the lights were switched on at the beginning of the civil twilight and off at dawn using an automatic time switch. The luminaires were equipped with 70 W HPS lamps (VIALOX NAV-T Super 4Y, yellow 2000 K, Osram, Munich, Germany) from 2012 until 2015. In the summer of 2015 these were replaced with 51W white LED lamps (TECEO 1, 32 LEDs, neutral white 4000K, Schréder, Brussels, Belgium) that produced light of comparable intensity at the water surface (19.5 ± 6.4 lux) to that of HPS lamps (17.1 ± 1.7 lux), directly in front of the lamps. Light intensity was measured with an ILT1700 underwater photometer (International Light Technologies Inc., Peabody, Massachusetts, USA), and the spectral composition was measured using a compact spectrometer (specbos 1211, JETI, Jena, Germany). The two control sites (Fig. 1) experienced a natural light regime with minimum nocturnal light levels of 0.002 ± 0.001 lux (mean and *SD*) for Control 1 (at 800m distance from the Lit site), and 0.010 ± 0.010 lux for Control 2 (at 300m distance from the Lit site). Light intensity was measured on clear nights during the new moon phase after astronomical twilight.

Experimental design and sampling procedures

For collection of periphyton, we constructed floating frames that held substrates for periphyton growth (Fig. 2a), submerged in the upper 15 cm of the water column in the middle of the ditch (Fig 2b). As substrates, we used plastic DIN A4 transparencies (polypropylene sheets with a slightly textured surface; PolyClearView, IBICO, GBC, Chicago, IL, USA), that were cut into strips (2 cm x 15 cm) that held together as a comb (Fig. 2a). The transparencies were fixed with metal clips to the floating frames and held in a vertical position facing the shore and the lamps (Fig 2b). These transparencies have been found to support the growth of periphyton communities similar to those growing on natural substrates such as macrophytes and muddy, organic sediments (Brothers *et al.*, 2013), that were also characteristic for this ditch. A total of 108 strips were deployed at each site. For each sampling, we randomly cut 4 replicate strips from transparencies at each site, avoiding strips with visibly clear paths in the periphyton indicative of grazing by snails. Strips were individually placed in 50-ml plastic screw vials filled with pre-filtered ditch water (Whatman GF/F glass-fiber filter, 0.7 µm nominal pore size, Whatman Ltd., Maidstone, UK) and stored on ice in darkness pending processing in the laboratory within 5 hours.

Two experiments with HPS lights were performed, in summer and winter 2014 (see Fig. 3 for details of the timeline). On 21 July and 28 October (Week 0), we deployed the frames with the substrates, and collected four 1-L water samples from the middle of the ditch at all three sites (see below). In summer, we collected strips with periphyton after 1, 2, 3 and 4 weeks of growth. In winter, the growth of periphyton was slower (likely due to low water temperatures), and the sampling was delayed to ensure that there was enough biomass for analysis. As a result, strips were collected after 5, 6, 7, 8 and 13 weeks of growth in winter. On 17 July 2015, HPS street lamps were replaced with LED lamps, and the experiment with LED lights was started following an initial acclimation period, on 11 November 2015 (Week 0, Fig. 3), when we installed new frames with the substrates and collected water samples.

Strips with periphyton were collected on a weekly basis from weeks 1 to 6, and again at week 13.

In all experiments, illumination of the treatment site started before periphyton exposure experiments began (Fig. 3). We therefore assessed potential chemical and biological differences between treatment and control sites at the beginning of each experiment by characterizing the water chemistry and phytoplankton community, each time we deployed experimental substrates. Water chemistry (carbon and nutrient levels, See Supplementary Material Table S1) was analyzed following standard chemical procedures (Krausse *et al.*, 1983, Murphy & Riley, 1962, Strickland & Parsons, 1968, Wetzel & Likens, 1991). Biomass and pigment composition of suspended phytoplankton that was available to serve as the founder community for the development of periphyton on the strips was assessed as described below (Table S2). Environmental parameters (temperature, pH, dissolved oxygen, and conductivity) were measured in time series, for each sampling event using a WTW Multi 3430 (WTW, Weilheim, Germany) equipped with WTW SenTix 940 pH sensor, WTW FDO 925 oxygen sensor, and WTW TetraCon 925 conductivity sensor (Tables S3-S5).

Laboratory procedures

Strips were removed from the 50-ml transportation vials. Periphyton was brushed from the strips with a toothbrush, and rinsed with pre-filtered ditch water (Whatman GF/F glass-fiber filter) into a graduated cylinder, to which the water used as transportation medium was also added to ensure that none of the biomass was lost. The total volume of the resulting suspension and the length of the strip that carried periphyton were recorded. After vigorous shaking, aliquots for determination of dry mass were concentrated on pre-combusted, pre-weighed 25 mm GF/F filters by vacuum filtration. Filters were dried at 65°C until constant mass was achieved, and re-weighed. Additional aliquots for pigment analysis were concentrated on filters and stored in 2-mL safety reaction vessels at -20°C pending analysis by high-performance liquid chromatography (HPLC) (Waters, Millford, MA, U.S.A). These

filters were transferred to -80°C for a minimum of 48 hours to stimulate cell lysis, and pigment composition was analyzed following the procedure described in Shatwell *et al.* (2012). All manipulations were performed under dim light to avoid pigment degradation. Dry mass and pigment composition of phytoplankton were analyzed from water samples following the same procedure.

Statistical analysis

Each light source and season (HPS in summer and winter, LED in winter) were analyzed separately. To test for effects of ALAN on periphyton biomass, we used generalized least squares linear models (GLS) (Zuur *et al.*, 2009) as available in the nlme package (Pinheiro *et al.*, 2015) for R (Version 3.1.3, R Development Core Team 2015). Site ("Lit", "Control 1", "Control 2"), and time (weeks) were included as factors in the model. Time series data were tested using the tseries package for R (Trapletti & Hornik, 2017). To account for temporal correlation, the model included an auto-regressive correlation structure of order 1 (Zuur *et al.*, 2009). Biomass values were log- or square root-transformed when necessary to improve distributional properties of the data. If variance heteroscedasticity was observed, the factors (site or time) were used as variance covariates (Zuur *et al.*, 2009). Pairwise comparisons were performed using the multcomp package for R (Hothorn *et al.*, 2008) with Benjamini-Hochberg corrections for multiple comparisons. To test for differences in the biomass of phytoplankton, GLS with site as factor was used, and post hoc comparisons were performed using *t* tests with Benjamini-Hochberg corrections.

Pigment composition was used as a composite indicator of periphyton community composition. Analysis of pigment composition, and especially pigment markers, is a commonly used method for quantification of algal classes in mixed communities (Jeffrey 1997). Patterns in pigment concentrations in relation to ALAN were analyzed by applying ordination analysis to all pigments identified in the samples. Pigment concentrations were normalized to chl *a*, z-standardized, and analyzed using principal component analysis (PCA)

with functions from the vegan (Oksanen *et al.*, 2015), and shape (Soetaert, 2014) packages for R. Pigment concentrations were log- or square root- transformed when necessary to meet the assumptions of normal distribution. The scores of the first PCA component that accounted for most of the variance in the data were statistically tested for effects of ALAN using the same GLS models as mentioned above, i.e. for periphyton including site and time as fixed factors and an auto-correlation structure; and for phytoplankton including site as fixed factor. Finally, we performed a correlation analysis of the PCA component scores, to determine which pigments were the drivers of variation in the data and the observed changes along the PCA axes.

Environmental parameters (measured in time series) were compared across all sites and three experiments by using GLS models with site as a factor, and an auto-regressive moving average (ARMA) correlation structure that allowed for modelling time series at sites for each experiment. When necessary, the variables were transformed and the model included a correlation structure (site, season). Chemical parameters (measured only once and transformed when needed) were compared among the sites for each experiment using one-way analysis of variance (ANOVA) or Welsch test (when heteroscedascity was observed), followed by tests for multiple comparisons with Bonferroni corrections.

R**esults**

Nocturnal HPS lighting

In summer, the biomass of periphyton that developed on the strips increased with time at all three sites (Fig. 4a. GLS: time effect $F_{1,41} = 76.53$, $p < 0.0001$). There were no differences in biomass between the sites (GLS: site effect $F_{2,41} = 0.64$, $p = 0.53$), and no significant interaction with time (GLS: site x time effect $F_{2,41} = 0.28$, $p = 0.75$).

Three chlorophyll pigments and five carotenoid pigments were identified in the periphyton (Table S6). PC1 accounted for 54% of variance in the pigment composition and

was correlated with violaxanthin (Pearson's r = - 0.93), lutein (r = - 0.92), chl *b* (r = - 0.87), and neoxanthin (r = - 0.87). PC1 clearly separated the Control 1 site from the two others (Fig. 5a), and PC1 scores indicated that periphyton pigment composition significantly changed over time (GLS: time effect $F_{1,42} = 5.53$, $p = 0.02$), and across all sites (GLS: site effect $F_{2,42} = 47.95$, $p < 0.0001$. *t* test: Lit to Control 1: $p < 0.0001$, Lit to Control 2: $p = 0.006$, Control 1 to Control 2: $p < 0.0001$). There was no significant interaction (GLS: site x time effect $F_{2,42} = 2.04$, $p = 0.15$).

In winter, the biomass of periphyton was highly variable (Fig. 4b) and changed significantly over time (GLS: time effect $F_{1,66} = 20.15$, $p < 0.001$), and across sites (GLS: site effect $F_{2,66} = 18.33$, $p < 0.0001$). The biomass at the Lit site did not differ from biomass at the Control 2 site (*t* test: $p = 0.22$), and both were significantly higher than that of Control 1 (*t* test: Lit to Control 1: $p < 0.0001$, Control 1 to Control 2: $p < 0.0001$). There was no significant interaction (GLS: site x time $F_{2,66} = 1.81$, $p = 0.17$).

The same eight pigments that were identified in the summer (above) were also identified in winter, along with one additional one (Table S7). PC1 accounted for 50% of the variance in pigment composition and was correlated with diadinoxanthin (Pearson's r = - 0.94), and lutein (r = - 0.86). Time-induced variation in pigment composition was visible along PC1 (Fig. 5b), separating week 4 from the rest of the samples. PC1 scores indicated that periphyton composition differed at all experimental sites (GLS: site effect $F_{2,54} = 5.93$, $p = 0.005$), and significantly changed over time (GLS: time effect $F_{1,54} = 15.22$, $p = 0.0003$). A significant interaction (GLS: site x time effect $F_{2,54} = 6.55$, $p = 0.003$) indicated that periphyton pigment composition changed in a different way across all sites over time.

Phytoplankton communities at the beginning of both HPS experiments did not show patterns clearly linked to ALAN exposure: biomass did not differ between the two closer sites (Lit and Control 2, apart by 300m from each other; mean and SD 4.1 ± 1.3 and 2.4 ±1.2 in summer, 39.6 ± 8.2 and 46.9 ±12,1 in winter, respectively; compared to 15.2 ± 4.2 at Control

1 in summer and 67.5 ±4.7 in winter; Table S8) despite the fact that the Lit site was exposed to artificial nocturnal illumination for over one year. Phytoplankton differed in pigment composition at all three sites (Tables S9-10).

Nocturnal white LED lighting

Following the transition of HPS to white LED lights, the biomass of periphyton was significantly affected by site (Fig. 4c, GLS: site effect $F_{2,78} = 18.43$, $p < 0.0001$), but not by time (GLS: time effect $F_{1,78} = 2.52$, $p = 0.12$). Starting from 3 weeks of growth until the end of the experiment (13 weeks), the biomass at the Lit site was significantly lower compared to Control 1 (*t* test: $p < 0.0001$), and Control 2 (*t* test: $p < 0.0001$) sites. The two control sites did not significantly differ from each other (*t* test: $p = 0.06$). There was no significant interaction (GLS: site x time effect $F_{2,78} = 1.08$, $p = 0.34$).

The same nine pigments that were identified in periphyton in previous winter experiment (HPS) were also identified for LED experiment (Table S11). PC1 accounted for 68% of the variance in pigment composition (Fig. 5c), and correlated with chl *c* (Pearson's $r = 0.94$), violaxanthin ($r = 0.94$), fucoxanthin ($r = 0.93$), and diadinoxanthin ($r = 0.93$). Scores of PC1 indicated different pigment composition of periphyton across all sites (GLS: site effect $F_{1,75} = 30.84$, $p < 0.0001$. *t* test: Lit to Control 1 $p < 0.0001$, Lit to Control 2 $p < 0.0001$, Control 1 to Control 2 $p < 0.0001$). There was no effect of time (GLS: time effect $F_{1,75} = 2.49$, $p = 0.12$), and no significant interaction (GLS: site x time effect $F_{1,75} = 0.54$, $p = 0.58$).

Phytoplankton communities at the beginning of the LED experiment did not show patterns that clearly linked to nocturnal light conditions: the biomass (mean and SD: Lit site 4.9 ±1.1, Control 1 17.0 ± 1.7, Control 2 8.6 ±1.8) and pigment composition differed from each other at all three sites, Table S9-10.

Discussion

We found no effect of nocturnal illumination by HPS lights on periphyton in either summer or winter, but following a replacement of the lights with white LEDs, the biomass of

periphyton that developed under a comparable nocturnal illumination (approx. 20 lux) in winter was up to 62% lower compared to periphyton that experienced a natural light regime. Neither light source had a substantial effect on periphyton community composition, as indicated by the relative pigment concentrations. This study is the first to report the effects of ALAN on periphyton in a lowland freshwater ecosystem, and partly confirm the findings reported for periphyton from a sub-alpine stream (Grubisic *et al.* 2017), namely that exposure to white LED light at night (approx. 20 lux) can decrease the biomass of freshwater periphyton in a natural setting. The biomass decrease can also be significant in communities from areas that experienced ALAN by HPS over multiple years, following a switch to LED lights. The type of the light source, likely its spectral composition and in particular the blue light component, seem to play a significant role in determining biological impacts of ALAN on periphyton biomass.

ALAN replaces the dark phase in a natural light/dark cycle, creating an environment with alternating phases of high-level natural light during the day and low-level artificial light during the night. Since algae are exposed to cycles of light and dark in their natural environment, and those have been stable over geological and evolutionary timescales, it is generally assumed that dark periods are necessary for their optimal growth (Carvalho *et al.*, 2011). Some species indeed require a dark period to obtain maximum growth rate (Zevenboom and Mur 1984). In many algal species DNA replication and cell division occur in darkness, and some phases of cell division cycle might be sensitive to light (Edmunds 1988). The interruption of a dark phase in a light/dark cycle by short periods of bright light was found to negatively affect growth of several species of diatoms and cyanobacteria (Gibson & Fitzsimons, 1991, Gibson & Fitzsimons, 1992, Zevenboom & Mur, 1984); although this has not been observed for all investigated species. In plants and moss, altered light regime has been shown to cause circadian stress, characterized by reduced photosynthetic efficiency and altered expression of clock genes (Nitschke *et al.*, 2016, Okada *et al.*, 2009). Change in

photoperiod imposed by ALAN can have a major impact on the disruption of circadian rhythms and physiology of plants (Kwak 2017). It is reasonable to assume that the disruption of a natural light regime by ALAN is likely to cause a distortion in circadian regulation of algae and cyanobacteria in periphyton as well.

Decreased biomass under similar levels of nocturnal white LED illumination was reported in several studies on algae and plants. Exposure to LED (20 lux, approx. 0.31 µmol m^{-2} s^{-1}) decreased the biomass of periphyton from a sub-alpine stream by up to 57% over 3 weeks (Grubisic *et al.* 2017), and 2 weeks of exposure (30 lux, approx. 0.44 µmol m^{-2} s^{-1}) decreased the biomass of bean plants by 22% (Sanders *et al.* 2015). By contrast, white LED light of a higher intensity (71 lux, approx. 1.3 µmol m^{-2} s^{-1}) was found to stimulate nocturnal photosynthesis in microbial communities (Hölker *et al.*, 2015). However, longer exposure (over 2 years) to higher level of nocturnal HPS light (approx. 82 lux, 1 µmol m^{-2} s^{-1}) was also found to decrease biomass of plants by 44-56% (Kwak *et al.* 2018). This decrease was associated with reduced photosynthetic efficiency and likely oxidative damage (Kwak *et al.* 2018).

The effect of ALAN on biomass of primary producers appears not to be linearly related to its intensity. While low-level light fall below the photosynthetic compensation point and have no effect on growth, and ALAN by LED of higher intensity can stimulate nocturnal photosynthesis, LED of intermediate intensities can have unexpected negative effects on biomass accrual. This may be caused by the interference of nocturnal illumination with the entrainment of the circadian clock and thereby the physiological and developmental processes that it regulates. The disruptive impact of nocturnal white LED light may be related to its blue component to which algae and plants are highly sensitive (Fig. 6). Light emitted by HPS lamps contains very little blue wavelengths; instead it is rich in yellow wavelengths that are not efficiently absorbed by photosynthetic pigments and cryptochromes (Fig. 6). This mismatch is likely to explain why no significant effect of nocturnal illumination by HPS on

periphyton communities was observed. For circadian disruption to occur under HPS illumination, higher light intensities may need to be applied at night (Kwak *et al.* 2018). Furthermore, low-level light that falls below the photosynthetic compensation point may still keep photosynthetic machinery active at night, which might be energetically costly (Poulin et al. 2014; Hölker et al. 2015).

ALAN by both HPS and LED illumination was previously reported to affect community composition of aquatic microbial communities, which is to be expected given a large variability in light optima and sensitivity between the taxa. Relative proportions of diatoms and cyanobacteria were altered by both short-term exposure (2-4 weeks) to mid-intensity LED illumination (20 lux, approx. 0.31 µmol m^{-2} s^{-1}) (Grubisic *et al.* 2017, 2018), and by long-term exposure (over 1 year) to low-level HPS illumination at night (6.8 – 8.5 lux, approx. 0.09 µmol m^{-2} s^{-1}) (Hölker *et al.* 2015). These effects were often season-dependent. In our study, there was a large variation in pigment composition of periphyton in all experiments, and no clear patterns could have been linked to nocturnal light conditions. It is possible that the detection of effects of ALAN on periphyton community composition was obscured by this variation. Even though pigment analysis can be a useful method for detection of ALAN effects on periphyton in outdoor mesocosm experiments (e.g. Grubisic *et al.* 2018), a method that provides better taxonomic resolution such as metabarcoding or microscopical identification may be more suitable for the field studies performed in more variable conditions.

Seasonal variation in environmental conditions, including light intensity and photoperiod, is not only an important driver of the taxonomic succession of periphyton communities, but also of their physiological acclimation to light (Biggs 1996, Falkowski and LaRoche 1991). As likely a consequence, the sensitivity and response of periphyton to ALAN varies with season (Grubisic *et al.* 2017, 2018). Here, effects of HPS were studied in two seasons (and season strongly influenced periphyton biomass, GLS: $F_{1,117} = 11.63$, $p < 0.01$),

but effects of LED illumination were studied only in winter. The seasonality in potential responses of periphyton to ALAN needs to be considered before drawing general conclusions on effects of LED on periphyton.

Apart from light, nutrients and grazers are also strong determinants of periphyton biomass (Stevenson, 1996). While environmental and chemical parameters showed seasonally variable patterns, most parameters did not differ between experimental sites (Tables S12-13), indicating similar conditions in the water across all sites. This was to be expected, given that the sites were located in one continuous ditch system (Fig. 1). Some parameters differed between the sites in the LED experiment (e.g. soluble reactive phosphorus, dissolved organic carbon, some nitrogen fractions and dissolved silica, Table S13), however, these differences are unlikely to explain the observed biomass decrease at the lit site, as the lowest levels of almost all of these parameters were recorded at one of the two control sites. Dissolved silica, although being lowest at the lit site, was of comparable levels to those measured in HPS experiment in summer, when no decrease in periphyton biomass was observed. This indicates that the biomass decrease observed at lit site under LED illumination cannot be explained by nutrient limitation. The grazers that were present in the system belonged to free-swimming invertebrates such as zooplankton and mayflies, and large grazers such as snails and fish (pers. obs.). Grazing activity was not measured in this study; however, strips that were grazed by snails were excluded from the analyses, and the abundance of free-swimming grazers in winter was very low based on observations from previous years (A. Manfrin, pers. comm.). Effects of grazing on periphyton were thus expected to be low in winter and unlikely to explain our results. Indirect effect of ALAN on periphyton through top-down control cannot, however, be excluded, especially in summer when grazers may be present in higher densities.

The experimental infrastructure was installed in 2012 in a rural and ALAN-naïve area (Holzhauer *et al.* 2015). By using this experimental setup we were able to separate effects of ALAN from other co-occurring factors typical of urban areas (e.g. increased nutrient levels,

chemical pollution, surface runoffs and sedimentation). The experimental sites were under the same management regime (e.g. grass-cutting, flow regulation) to minimize factors that might confound the effects of ALAN, thereby allowing for their quantitative comparison. By monitoring environmental variables we tried to capture potential effects of other important factors on periphyton. It is possible that the effects of HPS light were obscured by natural variability in other environmental factors, especially in the winter experiment when there was a large variation in the data (Fig.4, Table S4). Given the patterns we observed in the biological and environmental data at the beginning and during the course of each experiment, it is likely that ALAN was the strongest driver of the decline observed in periphyton biomass under LED light. As the use of LED illumination keeps increasing (Kyba *et al.* 2017a), its impacts on aquatic primary producers may be substantial not only in newly-lit areas (Grubisic *et al.* 2017) but also in areas that experienced light pollution by traditional lighting technologies over longer times. An ongoing transition to LED illumination may thus increase impacts of nocturnal illumination on periphyton.

Conclusions

Nocturnal low-level white LED illumination was previously reported to decrease the biomass of periphyton in a sub-alpine stream in spring and autumn (Grubisic *et al.*, 2017). Here, we found that the biomass decrease also occurred in winter, under a comparable LED illumination in a strongly contrasting environment, a lowland freshwater ecosystem. The reduced growth under nocturnal low-light white LED illumination (approx. 20 lux) may be a general response of periphyton to LED with a significant blue content. A better mechanistic understanding is needed to predict ecosystem consequences of different ALAN sources and light intensities, including the potential evolutionary adaptations and interactions with co-occurring factors in urban waters (see also Perkin *et al.* 2011). Periphyton is an important ecosystem component in streams, ponds, wetlands, and clear shallow lakes where it forms the

457 base of the food web and participates in biochemical cycling. The ongoing transition to LEDs
458 may increase ecological impacts of nocturnal illumination because decreased periphyton
459 biomass under white LEDs may potentially decrease nutrient turnover and food supply for
460 higher trophic levels, lowering production in the illuminated ecosystems.
461
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473
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669 **Figure Captions**

670 **Figure 1.** Aerial view of the study area located in Westhavelland Nature Park in
671 Brandenburg, Germany. Three experimental sites along a drainage ditch: Lit, Control 1 (C1)
672 and Control 2 (C2). Map data provided by Google, Digital Globe, GeoBasis-DE/BKG.

673 **Figure 2.** Scheme of the floating frames that were used to hold the substrates for
674 periphyton growth (transparencies cut in strips) (a), in a vertical position below the water
675 surface in the middle of the ditch (b).

676 **Figure 3.** Timeline of the experimental manipulations in three experiments performed
677 in two consecutive years under high-pressure sodium (HPS) and light-emitting diode (LED)
678 nocturnal illumination.

679 **Figure 4.** The biomass of periphyton (dry mass, mg cm^{-2}, mean and *SD*) developed at
680 the two control sites and at the Lit site, under high-pressure sodium lamps (HPS) in summer
681 (a, $n = 48$) and in winter (b, $n = 60$), or LED lamps in winter (c, $n = 84$).

682 **Figure 5.** Correlation biplots of principal component analysis (PCA) based on relative
683 pigment concentrations of periphyton, normalized to chlorophyll a, developed at the two
684 control sites and at the Lit site, under high-pressure sodium lamps (HPS) in summer (a, $n =$
685 48) and in winter (b, $n = 60$), or LED lamps (c, $n = 84$). Planes of the first two PC axes
686 explain 76% (a), 73% (b) and 89% (c) of the variation in the data. The pigments included in
687 the analysis were chlorophyll b (chl b), chlorophyll c (chl c), fucoxantin (fucox), violaxanthin
688 (violax), diadinoxanthin (diadinox), neoxanthin (neox), zeaxanthin (zeax), alloxanthin (allox),
689 and lutein. Arrows indicate correlation between original pigment variables and PC axes ($p <$
690 0.0001).

691 **Figure 6.** Spectral composition of the two light sources used in the study (high-
692 pressure sodium, HPS and light-emitting diodes, LED), spectral absorption curve of
693 chlorophyll *a* (based on Lohrenz *et al.*, 2003), and spectral sensitivity of cryptochromes
694 (based on Malhotra *et al.*, 1995).

Figure 1.

698 **Figure 2.**

699

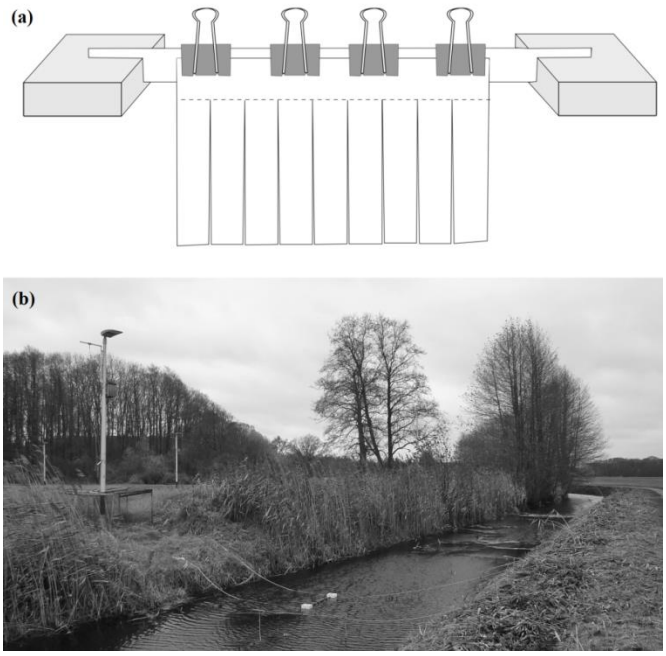

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Figure 3.

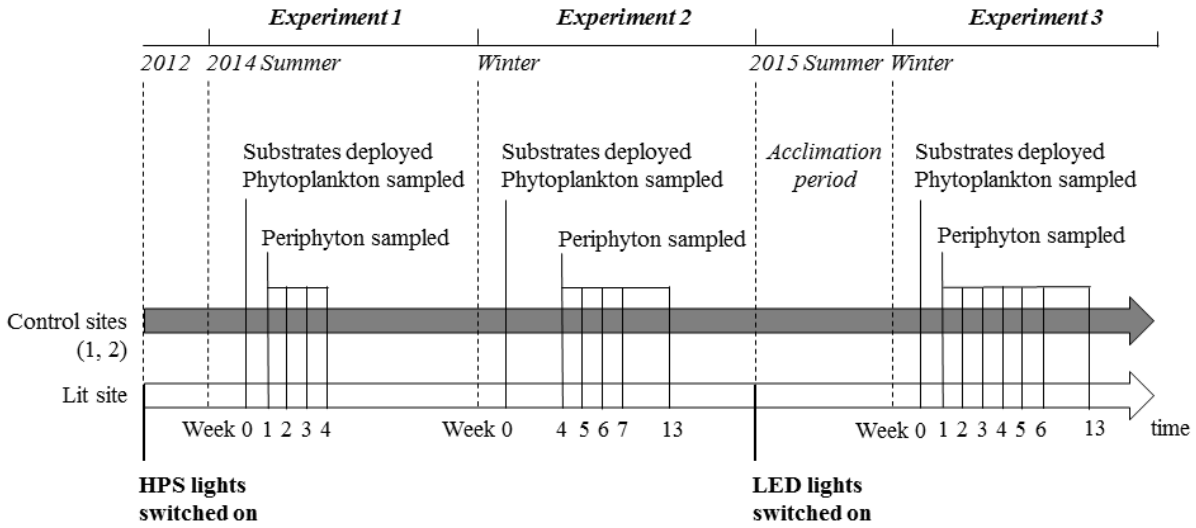

Experiment 1
Experiment 2
Experiment 3
2012
2014 Summer
Winter
2015 Summer
Winter
Substrates deployed
Phytoplankton sampled
Periphyton sampled
Substrates deployed
Phytoplankton sampled
Periphyton sampled
Acclimation period
Substrates deployed
Phytoplankton sampled
Periphyton sampled
Control sites (1, 2)
Lit site
Week 0 1 2 3 4
Week 0 4 5 6 7 13
Week 0 1 2 3 4 5 6 13
time
HPS lights switched on
LED lights switched on

Figure 4.

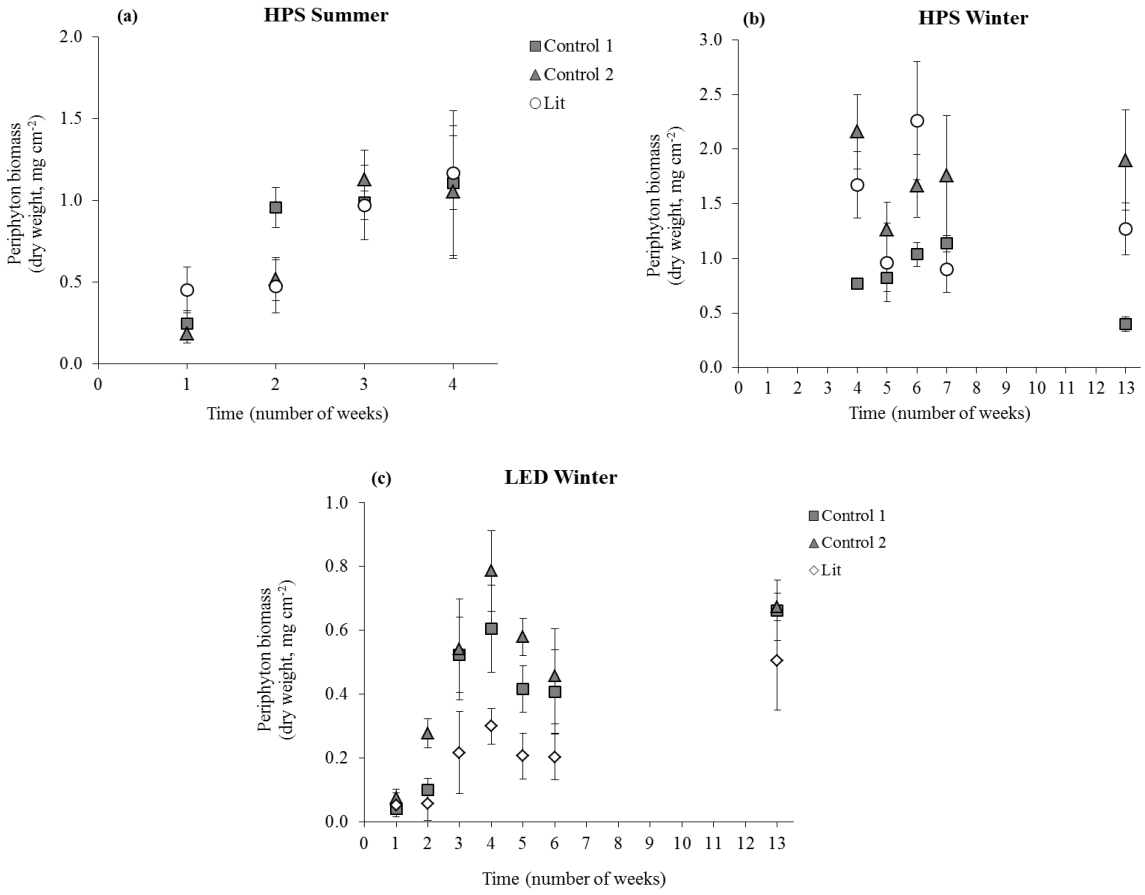
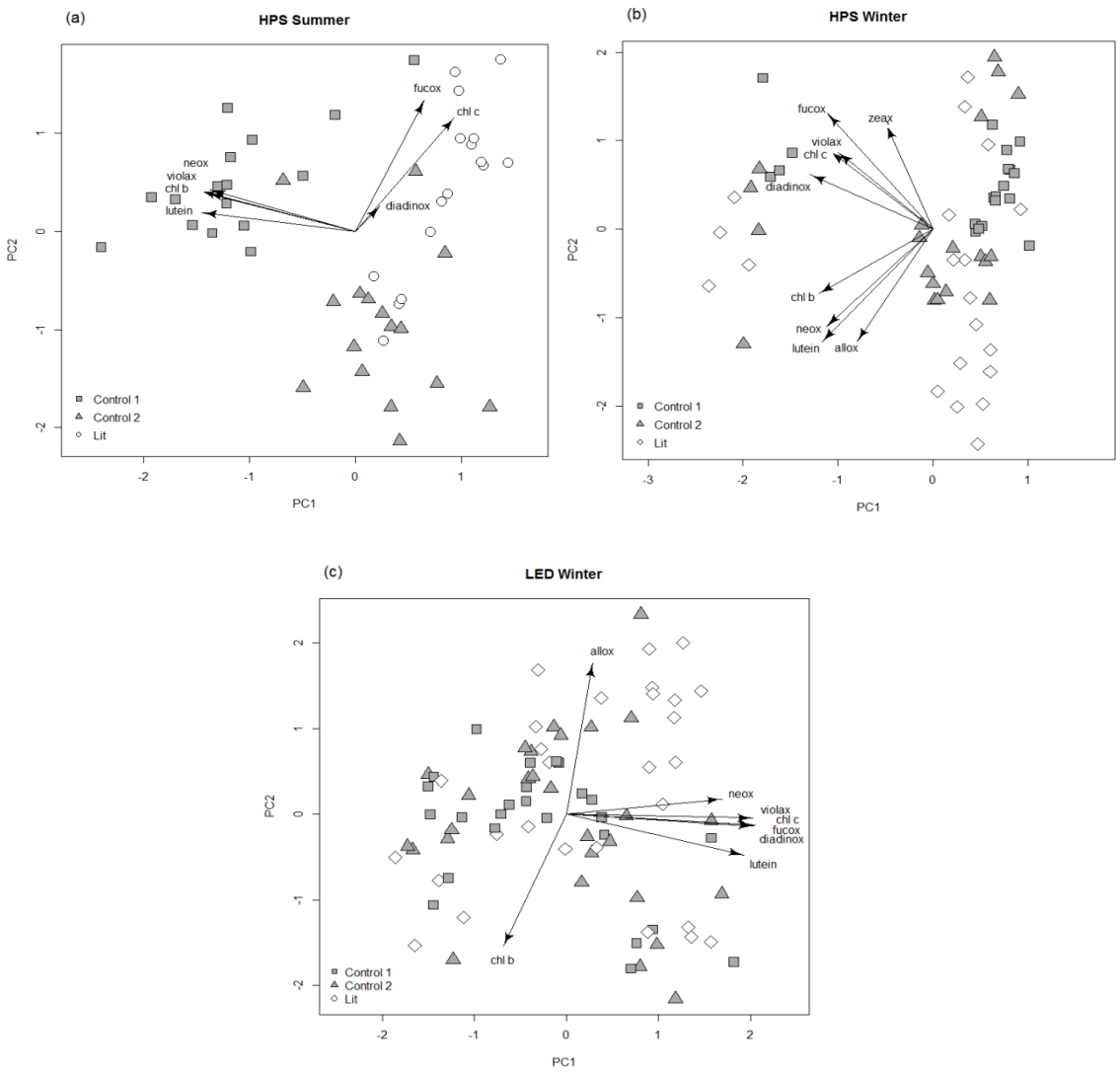
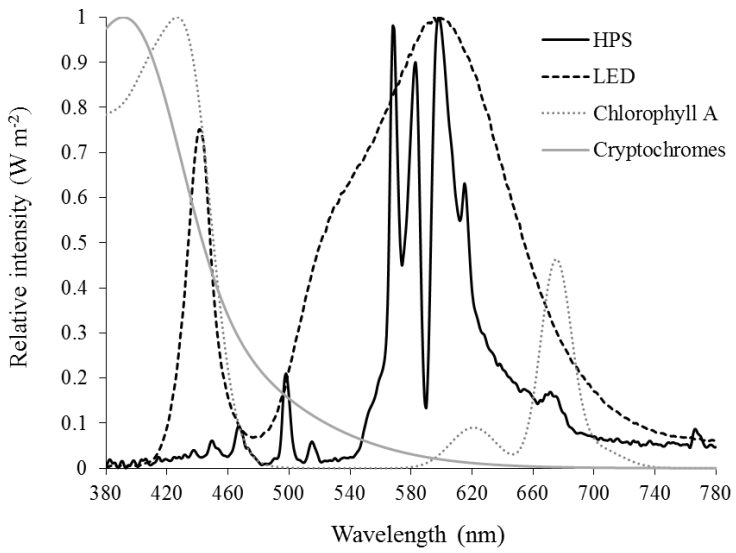

(a)
HPS Summer
Control 1
Control 2
Lit
Periphyton biomass (dry weight, mg cm-2)
Time (number of weeks)
(b)
HPS Winter
Periphyton biomass (dry weight, mg cm-2)
Time (number of weeks)
(c)
LED Winter
Control 1
Control 2
Lit
Periphyton biomass (dry weight, mg cm-2)
Time (number of weeks)

Figure 5.


(a)
HPS Summer
fucox
chl c
neox
violax
chl b
lutein
diadinox
Control 1
Control 2
Lit
PC1
PC2
(b)
HPS Winter
fucox
zeax
violax
chl c
diadinox
chl b
neox
lutein
allox
Control 1
Control 2
Lit
PC1
PC2
(c)
LED Winter
allox
neox
violax
chl c
fucox
diadinox
lutein
chl b
Control 1
Control 2
Lit
PC1
PC2

709 **Figure 6.**



710

Supplementary material for Grubisic *et al.* 2018 *"A transition to white LED increases ecological impacts of nocturnal illumination on aquatic primary producers in a lowland agricultural drainage ditch"*.

List of Tables

Site. Table shows estimates for factor Site and post hoc comparisons performed using *t* test with Benjamini-Hochberg corrections. Asterisk indicates a significant effect.

Table S10. Variance in the data explained by the first axis (PC1) calculated from principal component analysis based on relative pigment concentrations of phytoplankton at the beginning of three experiments. Table shows pigment that correlated with PC1 for each sampling and Pearson's correlation coefficient.

Table S11. Pigment composition of periphyton (µg cm^{-1}) developed at the three experimental sites over 1 to 13 weeks of substrate incubation for the winter sampling under light-emitting diode light (LED).

Table S12. Statistical analysis of environmental parameters measured with the multi probes at all experimental sites (Lit, Control 1 (C1) and Control 2 (C2)) compared across all three experiments performed under under high-pressure sodium (HPS) and light-emitting diode (LED) lights. Data analyzed using general least square model with Bonferroni correction for multiple comparisons. Asterisks indicate a significant difference.

Table S13. Statistical analysis of chemical parameters (in µg L^{-1}) measured at the three experimental sites (Lit, Control 1 (C1) and Control 2 (C2)) at the beginning of each of the three experiments performed under high-pressure sodium (HPS) and light-emitting diode (LED) lights. Asterisks indicate a significant difference.

Table S1. Chemical parameters (µg L^{-1}) measured at the three experimental sites at the beginning of each of the three experiments under high-pressure sodium (HPS) and light-emitting diode (LED) lights.

	HPS Summer			**HPS Winter**			**LED Winter**		
	Lit	Control 1	Control 2	Lit	Control 1	Control 2	Lit	Control 1	Control 2
Dissolved organic carbon	11.4	12.0	NA	10.3	10.0	NA	17.5	15.6	16.1
Dissolved nitrogen	0.70	0.79	NA	0.77	0.96	NA	1.75	1.56	1.63
Dissolved organic nitrogen	0.69	0.71	NA	0.59	0.64	NA	1.57	1.36	1.42
Nitrate-nitrogen	0.01	0.01	NA	0.01	0.01	NA	0.13	0.06	0.13
Nitrite-nitrogen	0.01	0.01	NA	0.01	0.01	NA	0.01	0.01	0.01
Ammonium-nitrogen	0.03	0.07	NA	0.17	0.31	NA	0.04	0.14	0.08
Solubile reactive phosphorus	24.7	63.0	NA	62.0	72.0	NA	15.0	40.0	11.0
Total phosphorus	94.0	132	NA	107.7	119.7	NA	63.0	75.3	64.6
Dissolved silica	3.27	6.12	NA	NA	NA	NA	4.83	6.07	5.27

Table S2. Pigment composition of phytoplankton (µg L^{-1}) developed at the three experimental sites under high-pressure sodium (HPS) and light-emitting diode (LED) lights.

	HPS summer			**HPS winter**			**LED winter**		
	Lit	Control 1	Control 2	Lit	Control 1	Control 2	Lit	Control 1	Control 2
Chlorophyll *a*	13.91	40.24	9.17	3.24	6.78	3.83	29.70	64.74	80.69
Chlorophyll *b*	2.97	10.59	1.25	n.d.	0.63	0.31	7.33	2.80	25.12
Chlorophyll *c*	0.29	0.68	0.33	n.d.	n.d.	n.d.	0.96	2.74	2.12
Fucoxanthin	0.64	2.17	0.46	0.25	0.65	0.18	2.72	10.93	6.90
Neoxanthin	0.17	0.78	0.05	n.d.	n.d.	n.d.	0.46	n.d.	1.26
Violaxanthin	0.33	0.66	0.09	n.d.	n.d.	n.d.	0.95	2.39	2.66
Diadinoxanthin	0.10	0.70	0.07	n.d.	0.21	n.d.	0.14	0.11	0.30
Alloxanthin	0.30	0.32	0.52	0.03	0.20	0.03	0.74	0.93	1.06
Lutein	0.58	2.01	0.21	n.d.	0.26	0.02	1.04	0.45	2.36
Zeaxanthin	0.08	0.48	0.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table S3. Environmental parameters measured with the multi probes at the three experimental sites for the summer sampling under high-pressure sodium lamps (HPS).

HPS summer	Lit					Control 1					Control 2				
time (weeks)	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Temperature (°C)	22.4	21.0	20.3	NA	16.8	26.7	23.4	21.7	NA	17.9	23.8	21.8	21.0	NA	17.0
Conductivity (µS cm^{-1})	506	468	530	NA	424	530	479	505	NA	645	486	435	434	NA	406
Oxygen (mg L^{-1})	5.34	6.11	6.43	NA	6.40	5.77	4.51	8.82	NA	8.22	10.28	12.56	12.70	NA	10.40
Oxygen (%)	62.8	69.4	72.2	NA	67.6	73.1	53.6	105.6	NA	88.7	123.5	148.4	150.3	NA	110.0
pH	7.5	7.6	7.5	NA	7.4	7.6	7.7	7.9	NA	7.9	7.6	8.2	8.4	NA	8.0

Table S4. Environmental parameters measured with the multi probes at the three experimental sites for the winter sampling under high-pressure sodium lamps (HPS).

HPS winter	Lit						Control 1					
time (weeks)	0	4	5	6	7	13	0	4	5	6	7	13
Temperature (°C)	11.3	3.5	3.2	3.0	2.9	3.1	11.6	3.4	2.4	2.0	2.5	3.2
Conductivity (µS cm^{-1})	500	516	538	526	551	468	553	557	603	558	722	545
Oxygen (mg L^{-1})	1.46	4.10	2.45	4.77	9.70	10.84	2.44	6.73	4.91	7.18	12.57	12.55
Oxygen (%)	13.3	31.3	18.1	36.7	72.6	82.8	22.4	50.4	35.7	52.4	93.2	96.3
pH	7.1	7.5	7.3	7.6	7.8	7.7	7.2	7.7	7.5	7.6	7.9	8.0

	Control 2					
time (weeks)	0	4	5	6	7	13
Temperature (°C)	NA	3.5	2.2	2.6	2.8	3.0
Conductivity (µS cm^{-1})	NA	506	535	532	558	485
Oxygen (mg L^{-1})	NA	5.06	2.68	5.20	11.00	11.00
Oxygen (%)	NA	38.0	19.3	38.5	82.6	84.1
pH	NA	7.6	7.2	7.4	7.8	7.7

Table S5. Environmental parameters measured with the probes at the three experimental sites for the winter sampling under light-emitting diode (LED) lights.

LED winter	Lit								Control 1							
time (weeks)	0	1	2	3	4	5	6	13	0	1	2	3	4	5	6	13
Temperature (°C)	10.6	10.4	2.0	5.5	7.6	4.7	7.8	5.9	10.8	10.4	2.8	5.6	7.5	4.7	7.9	5.8
Conductivity (µS cm^{-1})	545	534	500	556	513	515	498	454	573	586	617	622	652	621	611	536
Oxygen (mg L^{-1})	6.90	3.15	5.60	8.15	8.60	8.60	7.60	13.45	7.25	4.60	5.65	9.45	9.80	9.10	6.80	11.80
Oxygen (%)	63.0	28.3	40.6	64.5	70.4	66.2	63.7	110.8	66.0	42.0	42.8	74.5	80.3	69.8	57.5	97.2
pH	7.6	7.3	7.4	7.5	7.2	7.5	7.5	7.8	7.6	7.3	7.6	7.6	7.5	7.5	7.5	7.8

	Control 2							
time (weeks)	0	1	2	3	4	5	6	13
Temperature (°C)	10.5	10.4	2.6	5.6	7.8	4.7	7.9	5.9
Conductivity (µS cm^{-1})	540	540	549	567	561	513	516	467
Oxygen (mg L^{-1})	6.10	4.50	7.70	9.80	9.95	9.20	10.25	13.95
Oxygen (%)	54.7	40.5	59.2	78.0	82.5	70.9	86.2	114.9
pH	7.5	7.3	7.6	7.5	7.4	7.7	7.7	8.2

Table S6. Pigment composition of periphyton (µg cm^{-1}) developed at the three experimental sites over 1 to 4 weeks of substrate incubation for the summer sampling under high-pressure sodium lights (HPS).

HPS summer	Lit				Control 1				Control 2			
time (weeks)	1	2	3	4	1	2	3	4	1	2	3	4
Chlorophyll *a*	1.02	0.75	1.44	1.34	1.23	1.68	1.84	1.31	0.39	1.18	1.83	1.57
Chlorophyll *b*	0.18	0.11	0.11	0.17	0.41	0.67	0.64	0.41	0.08	0.19	0.25	0.35
Chlorophyll *c*	0.05	0.02	0.05	0.05	0.04	0.02	0.04	0.04	0.01	0.01	0.03	0.03
Fucoxanthin	0.12	0.05	0.18	0.15	0.13	0.11	0.16	0.07	0.03	0.04	0.11	0.11
Neoxanthin	0.01	0.01	0.02	0.00	0.02	0.05	0.04	0.04	0.00	0.01	0.02	0.02
Violaxanthin	0.01	0.01	0.02	0.02	0.03	0.05	0.05	0.03	0.01	0.02	0.03	0.03
Diadinoxanthin	0.02	0.01	0.03	0.03	0.01	0.01	0.02	0.02	0.05	0.01	0.02	0.02
Alloxanthin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lutein	0.02	0.03	0.05	0.05	0.05	0.13	0.12	0.08	0.01	0.04	0.07	0.06

Table S7. Pigment composition of periphyton (µg cm^{-1}) developed at the three experimental sites over 4 to 13 weeks of substrate incubation for the winter sampling under high-pressure sodium lights (HPS).

HPS winter	Lit					Control 1					Control 2				
time (weeks)	4	5	6	7	13	4	5	6	7	13	4	5	6	7	13
Chlorophyll *a*	6.44	2.62	4.03	3.56	5.68	2.54	1.28	2.21	4.49	2.18	6.54	2.84	4.18	4.60	6.99
Chlorophyll *b*	1.88	0.43	0.59	0.40	0.51	0.73	0.17	0.27	0.35	0.30	1.73	0.36	0.53	0.57	0.43
Chlorophyll *c*	0.91	0.13	0.23	0.27	0.29	0.50	0.10	0.17	0.38	0.16	0.95	0.21	0.34	0.33	0.44
Fucoxanthin	2.53	0.36	0.70	0.76	1.50	1.14	0.27	0.48	1.00	0.50	2.59	0.50	0.84	0.90	1.58
Neoxanthin	0.20	0.03	0.04	0.03	n.d.	0.05	n.d.	0.01	0.02	n.d.	0.15	n.d.	0.03	0.04	n.d.
Violaxanthin	0.16	0.03	n.d.	0.03	0.07	0.06	0.01	0.02	0.03	0.02	0.15	0.03	0.06	0.09	0.06
Diadinoxanthin	0.34	0.05	0.08	0.08	0.17	0.13	0.03	0.04	0.10	0.04	0.30	0.05	0.09	0.12	0.16
Alloxanthin	0.15	0.03	0.03	0.02	0.01	0.01	n.d.	n.d.	n.d.	n.d.	0.18	0.03	0.06	0.06	0.03
Lutein	0.38	0.08	0.11	0.07	0.06	0.08	0.02	0.03	0.04	0.03	0.30	0.05	0.09	0.10	0.06

Table S8. Statistical analysis of biomass of phytoplankton communities that were available as founder communities for development on periphyton at the three experimental sites (Control 1 (C1) and Control 2 (C2) and Lit) under high-pressure sodium (HPS) and light-emitting diode (LED) lights. Data analyzed using generalized least squares linear models with fixed factor Site. Table shows estimates for factor Site and post hoc comparisons performed using *t* test with Benjamini-Hochberg corrections. Asterisks indicate a significant effect.

		F	*df*	*p*		
HPS	Summer	25.25	1, 2	< 0.001*	*t* test:	Lit to C1 $p < 0.001$*
						Lit to C2 $p = 0.06$
						C1 to C2 $p < 0.001$*
	Winter	7.48	1, 2	0.01*	*t* test:	Lit to C1 $p = 0.01$*
						Lit to C2 $p = 0.29$
						C1 to C2 $p = 0.04$*
LED	Winter	62.48	1, 2	< 0.0001*	*t* test:	Lit to C1 $p < 0.001$*
						Lit to C2 $p < 0.001$*
						C1 to C2 $p = 0.002$*

Table S9. Statistical analysis of pigment composition of phytoplankton developed under high-pressure sodium (HPS) and light-emitting diode (LED) lights. Scores of the first axis (PC1) calculated with principal component analysis based on relative pigment concentrations were tested using generalized least squares linear models with fixed factor Site. Table shows estimates for factor Site and post hoc comparisons performed using *t* test with Benjamini-Hochberg corrections. Asterisk indicates a significant effect.

		F	*df*	*p*		
HPS	Summer	143.26	1, 2	< 0.0001*	*t* test:	Lit to C1 $p = 0.0001$*
						Lit to C2 $p < 0.0001$*
						C1 to C2 $p < 0.0001$*
	Winter	44.74	1, 2	< 0.001*	*t* test:	Lit to C1 $p < 0.001$*
						Lit to C2 $p < 0.001$*
						C1 to C2 $p = 0.55$
LED	Winter	13.56	1, 2	< 0.0001*	*t* test:	Lit to C1 $p = 0.03$*
						Lit to C2 $p = 0.002$*
						C1 to C2 $p = 0.03$*

Table S10. Variance in the data explained by the first axis (PC1) calculated from principal component analysis based on relative pigment concentrations of phytoplankton at the beginning of three experiments. Table shows pigment that correlated with PC1 for each sampling and Pearson's correlation coefficient.

		Explained variance (%)	Correlated pigments	Pearson's *r*
HPS	Summer	52	Lutein	- 0.99
			Chlorophyll *b*	- 0.93
			Neoxanthin	- 0.93
	Winter	52	Lutein	- 0.99
			Diadinoxanthin	- 0.96
LED	Winter	50	Alloxanthin	- 0.97
			Violaxanthin	-0.92

Table S11. Pigment composition of periphyton (µg cm^{-1}) developed at the three experimental sites over 1 to 13 weeks of substrate incubation for the winter sampling under light-emitting diode light (LED).

LED winter	Lit							Control 1							Control 2						
time (weeks)	1	2	3	4	5	6	13	1	2	3	4	5	6	13	1	2	3	4	5	6	13
Chlorophyll *a*	0.02	0.13	0.36	0.88	0.83	1.44	3.61	0.05	0.20	0.61	1.26	1.72	2.72	3.31	0.17	1.12	1.90	2.80	3.07	2.49	4.60
Chlorophyll *b*	n.d.	0.02	0.08	0.14	0.14	0.25	0.76	0.01	0.04	0.12	0.19	0.35	0.45	0.85	0.06	0.20	0.32	0.36	0.34	0.49	0.84
Chlorophyll *c*	n.d.	0.01	0.01	0.04	0.04	0.09	0.18	n.d.	0.01	0.03	0.07	0.10	0.19	0.15	n.d.	0.05	0.10	0.19	0.15	0.13	0.26
Fucoxanthin	n.d.	0.01	0.04	0.15	0.15	0.32	0.62	n.d.	0.02	0.09	0.25	0.35	0.71	0.53	0.01	0.13	0.27	0.65	0.62	0.56	0.89
Neoxanthin	n.d.	n.d.	n.d.	0.01	0.01	0.01	0.03	n.d.	n.d.	0.01	0.01	0.01	0.02	0.03	n.d.	0.01	0.02	0.02	0.02	0.09	0.03
Violaxanthin	n.d.	n.d.	0.01	0.01	0.01	0.02	0.03	n.d.	n.d.	0.01	0.02	0.02	0.03	0.03	n.d.	0.02	0.03	0.03	0.03	0.03	0.04
Diadinoxanthin	n.d.	n.d.	n.d.	0.01	0.01	0.03	0.06	n.d.	n.d.	0.01	0.02	0.03	0.05	0.04	n.d.	0.02	0.03	0.06	0.06	0.05	0.07
Alloxanthin	n.d.	n.d.	0.01	0.02	0.01	n.d.	n.d.	n.d.	0.01	0.02	0.01	0.01	n.d.	n.d.	n.d.	0.03	0.04	0.03	0.02	0.02	n.d.
Lutein	n.d.	n.d.	0.01	0.02	0.01	0.02	0.07	n.d.	n.d.	0.02	0.02	0.03	0.03	0.07	0.01	0.03	0.04	0.04	0.04	0.04	0.08

Table S12. Statistical analysis of chemical parameters (in µg L^{-1}) measured at the three experimental sites (Lit, Control 1 (C1) and Control 2 (C2)) at the beginning of each of the three experiments performed under high-pressure sodium (HPS) and light-emitting diode (LED) lights. Asterisks indicate a significant difference.

Comparisons between Lit and C1 site					*Comparisons between Lit, C1 and C2 sites*					
	Parameter	*F*	*df*	*p*		Parameter	*F*	*df*	*p*	*t test*
HPS Summer	SRP	146.57	1,6	< 0.001*	**LED Winter**	SRP	5406.9	2,3	< 0.001*	Lit to C1 p = 0.006*
	TP	2.3	1,5	0.18						Lit to C2 p < 0.001*
	NO3-N	Equal values across fields								C1 to C2 p = 0.005*
	NO2-N	Equal values across fields				TP	4.99	2,6	0.06	
	NH4-N	1,3	0.001			NO3-N	24.52	2,6	0.001*	Lit to C1 p = 0.003*
	DN	1,6	0.04							Lit to C2 p = 1
	DON	0.55	1,6	0.48						C1 to C2 p = 0.003*
	DOC	3.73	1,6	0.1		NO2-N	Equal values across all fields			
	Dsi	190.5	1,6	< 0.001*		NH4-N	7.4	2,6	0.02	Lit to C1 p = 0.03*
										Lit to C2 p = 0.54
HPS Winter	SRP	1.33	1,6	0.29						C1 to C2 p = 0.18
	TP	2.28	1,6	0.18		DN	2.79		0.14	
	NO3-N	Equal values across fields				DON	3.77	2,6	0.08	
	NO2-N	Equal values across fields				DOC	56.26	2,6	< 0.001*	Lit to C1 p < 0.001*
	NH4-N	240.1	1,6	< 0.001*						Lit to C2 p < 0.001*
	DN	34	1,6	0.001						C1 to C2 p = 0.13
	DON	3.5	1,6	0.11		Dsi	17.32	2,6	0.003*	Lit to C1 p = 0.003*
	DOC	0.79	1	0.37						Lit to C2 p = 0.22
	Dsi	NA								C1 to C2 p = 0.03*

Table S13. Statistical analysis of environmental parameters measured with the multi probes at all experimental sites (Lit, Control 1 (C1) and Control 2 (C2)) compared across all three experiments performed under under high-pressure sodium (HPS) and light-emitting diode (LED) lights. Data analyzed using general least square model with Bonferroni correction for multiple comparisons. Asterisks indicate a significant difference.

Parameter	*F*	*df*	*p*	*t test*	*F*	*df*	*p*	
Temperature (°C)	0.02	1,50	0.97					
Conductivity (µS cm^{-1})	8.9	2,50	< 0.001*	HPS summer	1.99	2,9	0.19	
				HPS winter	8.91	2,14	0.003*	Lit to C1 $p < 0.001$* Lit to C2 $p = 0.74$ C1 to C2 $p = 0.001$*
				LED winter	0.31	2,21	0.73	
Oxygen (mg L^{-1})	0.83	2,50	0.44					
Oxygen (%)	0.75	2,50	0.47					
pH	1.48	2,50	0.24					