Effects of artificial light at night on benthic primary producers in freshwaters

Inaugural-Dissertation to obtain the academic degree

Doctor of Philosophy (Ph.D.) in River Science

Submitted to the Department of Biology, Chemistry and Pharmacy of

Freie Universität Berlin

by

GRUBIŠIĆ MAJA

from Belgrade – Serbia

Berlin, 2017

The work on this doctoral thesis was conducted from 1st October 2013 to 30th March 2017 under the supervision of **PD Dr. Franz Hölker** (Leibniz Institute of Freshwater Ecology and Inland Fisheries Berlin) and **Dr. Michael T. Monaghan** (Leibniz Institute of Freshwater Ecology and Inland Fisheries Berlin), with co-supervision of **Dr. Maria Cristina Bruno** (Fondazione Edmund Mach, Italy) and **Prof. Dr. Guido Zolezzi** (University of Trento, Italy). The work was carried out at Leibniz Institute of Freshwater Ecology and Inland Fisheries Berlin, Germany, Freie Universität Berlin, Germany, University of Trento, Italy, and Fondazione Edmund Mach, Italy.

1st Reviewer: **PD Dr. Franz Hölker**

2nd Reviewer**: Prof. Dr. Klement Tockner**

Date of defence: 16th June 2017

The SMART Joint Doctorate Programme

Research for this thesis was conducted with the support of the Erasmus Mundus Programme, within the framework of the Erasmus Mundus Joint Doctorate (EMJD) SMART (Science for MAnagement of Rivers and their Tidal systems). EMJDs aim to foster cooperation between higher education institutions and academic staff in Europe and third countries with a view to creating centres of excellence and providing a highly skilled 21st century workforce enabled to lead social, cultural and economic developments. All EMJDs involve mandatory mobility between the universities in the consortia and lead to the award of recognised joint, double or multiple degrees.

The SMART programme represents a collaboration among the University of Trento, Queen Mary University of London and Freie Universität Berlin. Each doctoral candidate within the SMART programme has conformed to the following during their 3 years of study:

(i) Supervision by a minimum of two supervisors in two institutions (their primary and secondary institutions).

(ii) Study for a minimum period of 6 months at their secondary institution

(iii) Successful completion of a minimum of 30 ECTS of taught courses

(iv) Collaboration with an associate partner to develop a particular component / application of their research that is of mutual interest.

(v) Submission of a thesis within 3 years of commencing the programme.

This project has been funded with support from the European Commission. This publication reflects the views only of the author and the Commission cannot be held responsible for any use which may be made of the information contained therein

Mojim roditeljima

Table of contents

Summary

In recent decades, the use of artificial nocturnal illumination has rapidly increased worldwide, imposing an increase of nocturnal light levels and a disruption of natural cycles of light and dark that have been stable over geological and evolutionary time scales. This widespread alteration of the natural light regime by artificial light at night (ALAN) is contributing to global environmental change and raises concerns about the potentially adverse effects on organisms and processes in illuminated ecosystems. Simultaneously, a global shift in outdoor lighting technologies from yellow high-pressure sodium (HPS) to white light-emitting diode (LED) light is taking place, changing the spectral composition of nocturnal illumination. Mounting evidence suggests that ALAN affects microorganisms, plants and animals in both aquatic and terrestrial ecosystems. Light is a major source of energy and an important environmental cue for primary producers that influences and to a large extent drives their growth, production and community structure. Freshwaters are increasingly illuminated at night, as they are often located near the human population centers. Despite this, the impacts of artificial nocturnal illumination in freshwater ecosystems are still largely unknown. In particular, effects on aquatic primary producers in urban and sub-urban rivers and streams have hardly been addressed.

This thesis aimed to investigate effects of artificial nocturnal illumination on biomass and community composition of communities of benthic primary producers in freshwaters, the periphyton. The presented work is based on manipulative field studies performed in two contrasting freshwater systems whose periphyton communities are characterized by different species. The first study was performed in a stream-side flume system on a sub-alpine stream and the second in a lowland agricultural ditch. I found that two to 13 weeks of exposure to LED light at night decreased the biomass of periphyton in both aquatic systems. In stream periphyton, the decrease in biomass was observed for periphyton in early developmental stages (up to three weeks), but not that in the later developmental stages (four to six weeks). The effects of LED on community composition were found only in stream periphyton, where it increased the proportion of the dominant autotroph group, the diatoms and decreased the proportion of cyanobacteria in early developmental stages, but indicated a decreased proportion of diatoms and an increased proportion of cyanobacteria in the later developmental stages. I found that LED light at night altered pigment composition and quantitative taxonomic composition in stream periphyton in later developmental stages and that several diatom and chrysophyte taxa, both autotrophic and heterotrophic, responded to ALAN by increasing or decreasing in abundance

in a taxon-specific manner. LED did not affect periphyton community composition in lowland agricultural ditch, likely because periphyton was composed of different species. All effects of LED light were different between the seasons presumably due to seasonal differences in community composition and environmental variables. I did not find any evidence that HPS-light affects either biomass or community composition of periphyton. Differential effects of the two light sources are likely a result of differences in their spectral composition, in particular the high proportion of blue light emitted by LED but not by HPS.

This thesis provides, for the first time, evidence that LED light at night can profoundly affect benthic primary producers and periphyton communities in freshwater systems by reducing their biomass and altering community composition. Systems dominated by periphyton in its early developmental stages, such as streams prone to physical disturbances, are likely to be more sensitive to ALAN compared to systems with stable flow conditions based on the results presented. Periphyton plays a fundamental role in productivity, nutrient and carbon cycling and food supply for higher trophic levels in small, clear waters; its position in the base of aquatic ecosystems suggests that the alterations induced by ALAN may have important consequences for ecosystem functions. This should be considered when developing lighting strategies for areas close to freshwaters in order to mitigate potentially adverse effects of nocturnal artificial illumination on aquatic ecosystems.

Zusammenfassung

In den vergangenen Jahrzehnten hat die Verwendung künstlicher Beleuchtung in der Nacht weltweit rasant zugenommen. Diese Anhebung des natürlichen nächtlichen Lichtniveaus stört Hell-/ Dunkel- Zyklen, welche bisher geologisch wie evolutionäre stabil waren. Diese oft flächendeckenden Veränderungen des natürlichen Lichtregimes durch künstliches Licht in der Nacht ist eine globale Veränderung der Umweltbedingungen, welche Fragen aufwerfen über mögliche negative Auswirkungen auf Organismen und Prozesse in beleuchteten Ökosystemen. Außerdem verändert sich derzeit die spektrale Zusammensetzung der nächtlichen Beleuchtung durch moderne Technologien von gelblich scheinenden Natrium-Hochdruck-Lampen (HPS) hin zu weißen Leuchtdiode (LED). Mehr und mehr Studien weisen nach, dass künstliche Beleuchtung in der Nacht Mikroorganismen, Pflanzen und Tiere sowohl in terrestrischen als auch in aquatischen Ökosystemen beeinflusst. Licht ist eine wichtige Energiequelle und ein Signal für Primärproduzenten, es beeinflusst ihr Wachstum, ihre Reproduktion und ihre Gemeinschaftsstruktur. Auch Binnengewässer werden nachts zunehmend beleuchtet, da sie sich oft in unmittelbarer Nachbarschaft zu Wohnsiedlungen befinden. Trotzdem sind die Auswirkungen durch künstliche Beleuchtung in der Nacht auf Süßwasserökosysteme noch weitestgehend unbekannt. Insbesondere Auswirkungen durch Beleuchtung in urbanen und peri-urbanen Flüssen auf aquatische Primärproduzenten wurden bisher kaum beachtet.

Diese Dissertation behandelt die Auswirkungen durch künstliche Beleuchtung in der Nacht auf die Biomasse und die gemeinschaftliche Zusammensetzung der Süßwasser-gemeinschaften benthischer Primärproduzenten des Periphytons. Die manipulativen Freilandexperimente wurden in zwei unterschiedlichen Süßwassersystemen durchgeführt, welche sich durch unterschiedliche Periphytongemeinschaften auszeichnen. Das erste Experiment wurde in einem Rinnensystem an einem subalpinen Fluss durchgeführt, das zweite Experiment in einem landwirtschaftlich genutzten Grünlandentwässerungssystem im Flachland. Ich fand heraus, dass 2 - 13 Wochen Expositionszeit unter LED-Beleuchtung die Biomasse der Primärproduzenten des Periphytons beider aquatischer Systeme verringerte. Im Periphyton des subalpinen Flusses beobachtete ich Auswirkungen auf die Biomasse des Periphytons in frühen Entwicklungsstadien (bis zu drei Wochen), aber nicht in den älteren Stadien (vier bis sechs Wochen). Diese Auswirkungen der LED-Beleuchtung wurden nur für die Gemeinschaftszusammensetzung des Periphytons im Fluss nachgewiesen. Der Anteil der

dominanten Autotrophe, der Kieselalgen, wuchs in den ersten Entwicklungsstadien an und der Anteil der Cyanobakterien nahm ab, in den späteren Stadien zeichnete sich aber ein reduzierter Anteil an Kieselalgen ab und der Anteil der Grünalgen nahm zu. Ich habe festgestellt, dass LED Beleuchtung die Pigmentzusammensetzung und die quantitativen taxonomischen Gemeinschaftsverhältnisse in älteren Entwicklungsstadien des Periphytons im Fluss verändert und dass mehrere Kieselalgen und Chrysophyten Taxa, sowohl autotrophe wie auch heterotrophe, empfindlich auf künstliches Licht reagieren, indem sie taxon-spezifisch entweder zu- oder abnehmen. Die LED-Beleuchtung zeigte keinen Einfluss auf die Periphyton-Gemeinschaft des Grünlandgrabens, wahrscheinlich wegen der unterschiedlichen Artengemeinschaft. Alle Effekte waren stark saisonabhängig, vermutlich wegen der saisonalen Gemeinschaftszusammensetzungen und der Umweltvariablen. Ich habe keine Nachweise dafür gefunden, dass HPS-Licht die Biomasse oder die Gemeinschafts-zusammensetzung des Periphyton beeinflusst. Die abweichenden Ergebnisse der beiden Lichttechnologien sind wahrscheinlich der unterschiedlichen spektralen Zusammensetzung geschuldet, insbesondere dem hohe Blaulichtanteil der LED-Beleuchtung, welcher von HPS-Lampen nicht ausgestrahlt wird.

Die vorliegende Studie führt das erste Mal den nachweis, dass LED-Beleuchtung die benthischen Primärproduzenten und Periphytongemeinschaften in Süßwassersystemen beeinflussen kann, indem die Biomasse abnimmt und die Gemeinschaftsstrukturen verändert werden. Systeme, die von frühen Periphyton Entwicklungsstadien dominiert werden, wie solche, die oft gestört physisch werden, reagieren wahrscheinlich empfindlicher auf künstliches Licht in der Nacht als stabile Systeme, basierend auf den hier dargestellten Ergebnissen. Periphyton spielt eine wesentliche Rolle bei der Produktivität von Nähr- und Kohlenstoffzyklen und damit für die Nahrungsmittelversorgung höherer Trophieebenen in kleinen, klaren Gewässern. Wegen der Stellung des Periphytons an der Basis der aquatischen Ökosysteme kann davon ausgegangen werden, dass durch künstliches Licht in der Nacht hervorgerufene Veränderungen wichtige Konsequenzen für Ökosystemfunktionen haben. Diese Ergebnisse sollten bei der Planung von Beleuchtung in gewässernahen Gebieten berücksichtigt werden, um mögliche nachteilige Auswirkungen durch Beleuchtung auf aquatische Ökosysteme zu vermeiden.

Thesis outline

This thesis is composed of four manuscripts that are either published, submitted for publication or ready to be submitted to peer-reviewed journals. Each manuscript forms a chapter of the thesis (Chapters 2 to 5) and contains an introduction, methodology, results, discussion and conclusion sections as well as bibliography. In the general introduction (Chapter 1) I provided the context for the thesis, general research aims and aims of the individual chapters. In the general discussion (Chapter 6) I connected the main findings of the individual chapters, discussed them in a broader context and highlighted the potential for the further research.

Chapter 1:

General introduction

Chapter 2:

M. Grubisic, G. Singer, M.C. Bruno, R.H.A. van Grunsven, A. Manfrin, M.T. Monaghan, F. Hölker (published) Artificial light at night decreases biomass and alters community composition of benthic primary producers in a sub-alpine stream.

Modified article published in Limnol. Oceanogr., 2017. http://dx.doi.org/10.1002/lno.10607

Author's contributions: **MG**, MCB, MTM and FH designed the study. **MG**, MCB and AM collected the data. **MG** and GS performed statistical analysis. **MG**, GS and RHAVG interpreted the data and conceptualized the manuscript. **MG** wrote the manuscript and all co-authors contributed to the text.

Chapter 3:

M. Grubisic, G. Singer, M.C. Bruno, R.H.A. van Grunsven, A. Manfrin, M.T. Monaghan, F. Hölker (in review) A pigment composition analysis reveals community changes in stream periphyton under low-level artificial light at night.

Article submitted to Limnologica on 24. May 2017.

Author's contributions: **MG**, MCB, MTM and FH designed the study. **MG**, AM and MCB collected the data. **MG** analyzed the HPLC spectrograms. **MG** and GS performed statistical

analysis. **MG**, GS and RHAVG interpreted the data. **MG** wrote the manuscript and all co-authors contributed to the text.

Chapter 4:

M. Grubisic, J. Zimmermann, C. Wurzbacher, S. Proft, F. Hölker, M.T. Monaghan (in prep.) DNA metabarcoding the 18S rRNA V4 region to investigate effects of artificial light at night on stream periphyton.

Author's contributions: **MG**, MTM and FH designed the study. **MG** collected the data and performed laboratory analyses. JZ and MTM contributed to data production and interpretation. JZ and SP performed bioinformatics analyses. **MG** and CW performed statistical analysis. **MG** wrote the manuscript and all co-authors revised the text.

Chapter 5:

M. Grubisic, R.H.A. van Grunsven, A. Manfrin, M.T. Monaghan, F. Hölker (in prep.) Light source matters: Nocturnal low-light LED illumination decreases periphyton biomass, but high-pressure sodium does not.

Author's contributions: **MG**, MTM and FH designed the study. **MG** collected the data and analyzed the HPLC spectrograms. **MG** and AM performed statistical analysis. **MG** and RHAVG conceptualized the manuscript. **MG** wrote the manuscript and all co-authors contributed to the text.

Chapter 6:

General discussion

List of figures

Supplementary figures

List of tables

Supplementary tables

1. General introduction

1.1. Distribution of artificial light at night and implications for freshwater ecosystems

Artificial light at night (ALAN) is considered to be one of the fastest-spreading human-induced environmental alterations that is contributing to global change (Falchi et al. 2016; Hölker et al. 2010a). The use of artificial sources of light is increasing natural nocturnal light levels over 23% of the world's land surfaces between 75°N and 60°S, including 88% of Europe and almost 50% of the United States (Falchi et al. 2016). This is a relatively novel environmental pressure associated with the increasing use of electrical lighting in the last decades that influences physiology and behavior of organisms and acts as an important driver of ecosystem dynamics (Gaston et al. 2013; Hölker et al. 2010a; Rich and Longcore 2006).

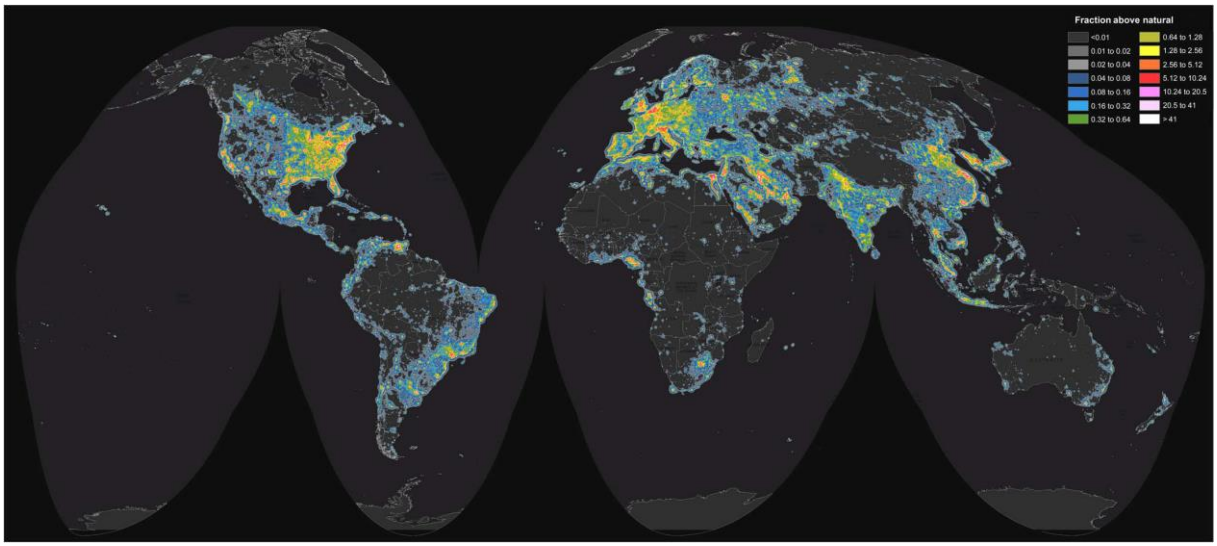

Figure 1. World map of artificial sky brightness (Falchi et al. 2016)

Freshwaters are increasingly illuminated at night worldwide. Human settlements are often associated with coastal and riparian zones (Lowe and Pan 1996) because easy access to freshwater was essential for the development of human civilizations. Abundant freshwater supplies are necessary for human consumption and food production and important for industry and transportation (Lowe and Pan 1996). The human presence close to freshwaters has been consistently increasing around the globe inducing drastic environmental changes (Ceola et al. 2015). More than half of the world's population currently lives within 3 km of a body of freshwater (Kummu et al. 2011). As a result of human population growth, increasing urbani-

zation and spread of electrical lighting, freshwaters that previously did not experience artificial nocturnal illumination, are being increasingly exposed to artificial light at night worldwide.

Biological and ecological implications of artificial nocturnal illumination have received increasing attention from the scientific community over the last decade. Extensive discussions on potential effects of ALAN for ecosystems and their inhabitants (Navara and Nelson 2007; Rich and Longcore 2006) have drawn attention to the topic. An effort has been put into synthesizing available knowledge, identifying research gaps and developing frameworks for further research (Gaston et al. 2013; Gaston et al. 2015; Hölker et al. 2010a; Perkin et al. 2011). ALAN may act as a global biodiversity threat (Hölker et al. 2010b; Kyba and Hölker 2013) and the empirical evidence documenting its effects on microorganisms, plants, animals and humans is rapidly mounting. Research in freshwater ecosystems and aquatic ecosystems in general, is however lagging behind the research conducted in terrestrial systems (see Gaston et al. 2014; Perkin et al. 2011). In particular, aquatic primary producers have rarely been studied (e.g. Hölker et al. 2015; Poulin et al. 2014), probably because it is often considered that underwater light levels resulting from street illumination and other urban light sources are too low to significantly affect photosynthesis in aquatic primary producers.

1.2. Importance of light for primary producers

Light is a fundamental source of energy and an important source of information for primary producers (Hegemann et al. 2001). It is a prerequisite for autotrophic existence that provides energy necessary to synthesize biomass from inorganic compounds in the process of photosynthesis. The properties of light, such as intensity, spectral composition, timing and duration, all influence photosynthesis and growth of aquatic primary producers (Falkowski and LaRoche 1991; Richardson et al. 1983). Light is also a source of information about the external environment, necessary for entrainment of the internal, circadian clock. The regular cycle of light and dark, driven by the Earth's rotation, is one of the most regular and stable environmental fluctuations that have been stable over geological and evolutionary time scales. Many organisms, including primary producers, have evolved sensitive receptors to perceive light rhythms and through complex pathways of circadian regulation use this information to drive rhythmic changes in biological processes to synchronize with their environment (Brand and Guillard 1981; Fortunato et al. 2015 and references therein). By illuminating the nights with artificial light, humans alter the natural intensity and timing of light, increasing nocturnal

light levels by several orders of magnitude during what would naturally be a dark period (Gaston et al. 2013). The quality of light that is available at night deviates from that of moonlight or starlight, because the distinctive spectral signatures of sources of artificial light differ significantly from those of natural light (Gaston et al. 2013). The disruption of natural light regime by ALAN might therefore influence physiology of primary producers in complex and multiple ways, rather than only influencing photosynthesis.

1.3. Benthic primary producers and periphyton: distribution, roles and community dynamics in freshwaters

Benthic primary producers, algae and cyanobacteria, grow attached to submerged surfaces reached by light, forming complex periphyton communities and are fundamental components of many aquatic systems (Stevenson 1996; Wu 2017). They are embedded in a mucopolysaccharide matrix together with heterotrophic bacteria, fungi, protozoa and detritus. Periphyton is ubiquitous, occurring in almost all aquatic ecosystems (Wu 2017), developing on all types of submerged surfaces including hard and soft substrates and aquatic vegetation. Benthic primary producers are the main component of periphyton; in freshwaters they are represented predominantly by diatoms as well as green algae, cyanobacteria and to a smaller extent red algae, chrysophytes and euglenophyta (Stevenson 1996). Periphyton is usually species-rich and responds rapidly and sensitively to variations in physical, chemical and biological parameters and therefore is commonly used for assessing environmental conditions in aquatic environments in biological monitoring (Lowe and Pan 1996; Smol and Stoermer 2010). Periphyton forms the foundation of aquatic food webs, plays a key role in nutrient and carbon cycling and provides habitat for many organisms (Law 2011; Stevenson 1996); it often dominates primary production in clear shallow waters and small to mid-sized streams (Dodds et al. 1999). Benthic primary producers occupy a pivotal position in aquatic ecosystems at the interface of the chemical-physical and biotic components of the food web. This is a critical link in aquatic ecosystems and its disruption can profoundly influence the rest of the aquatic community (Lowe and Pan 1996).

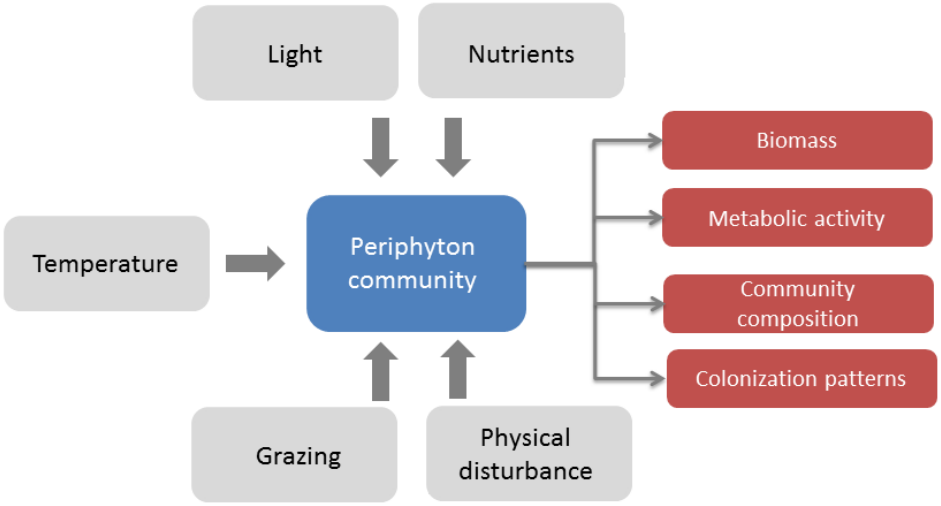

Figure 2. Proximate factors that regulate dynamics of periphyton communities in aquatic systems

Light interacts with other abiotic and biotic factors to determine periphyton community composition (Figure 2). Light and nutrients are fundamental resources necessary for existence and survival of primary producers and proximate factors that directly regulate biomass accrual through bottom-up effects (Biggs 1996). Biomass loss is mainly regulated by top-down effects such as physical disturbances, e.g. high flow velocity, substrate instability and perturbation, and biological factors i.e. grazing. Temperature influences metabolism, growth rates and rates of enzyme activity and thus modulates effects of resources. All these factors interact to determine the biomass, composition and structure of periphyton communities (Biggs 1996) as well as their nutritional quality for herbivores (Guo et al. 2016). Aquatic herbivores, macroinvertebrates such as larvae of aquatic insects and snails, and herbivore fish, can be important determinants of biomass, structure and composition of periphyton (Steinmann 1999). Light interaction with grazing has been extensively studied under daylight conditions, but much less is known about their interaction at night. Activity and movement in aquatic insects is suppressed by natural and artificial light at night (Bishop 1969; Elliott 1968), but the extent to which this affects the feeding rates of these grazers on periphyton is not known.

1.4. Importance of light for periphyton communities

Disruption of the natural light regime by ALAN may impact periphyton communities, because variations in light intensity, quality and availability are known to have profound ef-

fects on the physiology, structure and growth of periphyton (Wu 2017) as well as its taxonomical and biochemical composition (Cashman et al. 2013; Falkowski and LaRoche 1991). Autotrophic species within periphyton have markedly different photosynthetic optima and minimum light requirements necessary for growth (Richardson et al. 1983). All major taxonomic categories contain species that are able to grow in very low light environments; however, cyanobacteria and in particular diatoms are generally considered to be better adapted to grow under low light levels compared to green algae (Langdon, 1988; Richardson et al., 1983). Within each of these categories, there is a considerable variability between the taxa in the ability to use of low light levels that is driven by differences in amount and types of pigments and physiognomy (Hill 1996; Lange et al. 2011). These differences might drive differential responses of periphytic taxa to artificial nocturnal illumination and result in altered taxonomic composition and diversity of periphyton under ALAN. The changes in species composition can affect structural and functional properties of periphyton, which may then be reflected at the whole ecosystem level (Wu 2017).

Sensitivity and response of periphyton to artificial nocturnal illumination may vary between different colonization stages. Under stable flow conditions, the development of periphyton follows characteristic colonization patterns that include biomass accrual, succession in species and growth forms and a change in the vertical structure of the community (Biggs 1996). Adsorption of nutrients and dissolved organic substances onto submerged surfaces allows primary colonization by bacteria. Secondary colonization starts with settling of small low-profile, adnate diatoms followed by high-profile, stalked and erected taxa of larger pennate diatoms, cyanobacteria and protozoa. In the tertiary colonization phase, high-profile diatoms become dominant, together with filamentous green algae and complex multicellular taxa (see Biggs 1996; Wu 2017). Biomass accrual over time thus leads to the establishment of three-dimensional, spatially complex biomass matrices (Biggs 1996), while succession drives a shift in growth forms from the dominance of low-profile diatoms towards complex high-profile forms (Biggs 1996; Hudon and Bourget 1983). The development of this microscale architecture is strongly influenced by light conditions; high-profile taxa have competitive advantage for light over low-profile taxa (Hudon and Bourget 1983). As light gets absorbed by pigments in overlying cells and attenuated in the matrix, light availability is closely related to periphyton vertical structure and diversity of growth forms (Hudon and Bourget 1983). Vertical development of the periphyton matrix thus modifies the environmental conditions within the matrix itself, modulating a community response to light (Boston and Hill 1991). A re-

sponse of periphyton to ALAN may thus also be expected to vary between communities at different colonization stages.

The ability of periphyton to detect and respond to relatively low-light levels produced by ALAN may differ between the seasons. Variations in photosynthesis and growth rates of algae in nature are largely determined by their ability to adapt to daily and seasonal changes in intensity and spectral composition of light through processes of physiological acclimation (Falkowski and LaRoche 1991). By changes in cellular and pigment composition algae maximize photosynthetic efficiency under alailable light. In temperate latitudes, autotrophs are generally better adapted to lower light conditions in winter and spring and higher light conditions in summer and autumn (Kirk 1994; Laviale et al. 2009); therefore taxa may also respond more strongly to low-light levels of ALAN in winter and spring. Furthermore, seasonal differences in environmental conditions, in particular light intensity and photoperiod, strongly drive the succession of periphyton communities (Biggs 1996) and may reflect in their photosynthetic capacity. Thus seasonal differences in taxonomic composition may affect periphyton sensitivity and response to ALAN.

1.5. Potential effects of artificial light at night on primary producers

Both intensity and spectral composition of light are important properties that may determine the effect of ALAN on photosynthesis and circadian regulation in primary producers. Nocturnal artificial illumination may stimulate photosynthesis at times when it does not naturally occur (Aube et al. 2013; Gaston et al. 2013); however the intensity of illumination produced by outdoor lighting (typically not more than 0.5 µmol m^{-2} s^{-1}) is extremely low compared to daylight (from 100 µmol m^{-2} s^{-1} in shaded environments to 2000 µmol m^{-2} s^{-1} in sunlit conditions) (Gaston et al. 2013). Therefore the effect of outdoor lighting on net photosynthetic production is expected to be low, with the exception of directly illuminated areas close to the light sources (Gaston et al. 2013; Raven and Cockell 2006). Whether light levels in urban and suburban areas are high enough for net photosynthesis, has not been studied. The minimum light intensity needed for photosynthesis of microalgae is not accurately determined in the literature, but is considered to be below 1 µmol $m^{-2}s^{-1}$ (approx. 50 lux) based on observations (Richardson et al. 1983). The theoretical minimum needed for the photosynthesis lies between 0.01 µmol m^{-2} s^{-1} (approx. 0.5 lux) and 0.1 µmol m^{-2} s^{-1} (approx. 7.4 lux), slightly above the light level of a full moon on a clear night (0.005 µmol m^{-2} s^{-1}, approx. 0.3 lux) (Raven and Cockell 2006). Photoreceptors, however, detect intensities far below the sensitivity

thresholds for photosynthesis, including moonlight (approx. 0.1 lux) (Bünning and Moser 1969; Petroutsos et al. 2016), therefore these light levels can affect other processes in algae. The dark/light regime influences many cellular, physiological and developmental processes, growth and biomass production in primary producers (see Fortunato et al. 2015; Hegemann et al. 2001) often displaying circadian and even lunar periodicity (Devlin and Kay 2001; Schad 1999). The disruption of natural light regime and light/dark cycles even by low-intensities of light might therefore disrupt mechanisms of internal timekeeping and affect processes regulated by circadian rhythms.

ALAN rich in wavelengths efficiently absorbed by photosynthetic pigments is more likely to stimulate nocturnal photosynthesis (Aube et al. 2013). Light utilized in photosynthesis, photosynthetic active radiation, falls within the visible spectrum and ranges from 400 to 700 nm; but not all of these wavelengths are utilized as efficiently. Chlorophylls, the main photosynthetic pigments found in all photosynthetic organisms, most efficiently absorb blue (450 - 475 nm) and red light (630 - 675 nm). Accessory pigments such as carotenoids play only a minor role in photosynthetic processes; they absorb light in violet/blue and blue/green region (400 - 550 nm) and transfer some of its energy to the chlorophylls. Carotenoids mainly absorb excess light and are thus involved in photoprotection. Phycobillins are photosynthetic pigments found only in cyanobacteria and absorb green, yellow and orange light (500 - 650 nm). Green and yellow wavelengths are not efficiently absorbed by chlorophylls nor utilized in the photosynthetic processes, but they convey important information for developmental processes (Yeh and Chung 2009). Photoreceptors detect light across the visible spectrum (Rockwell et al. 2014) and blue light (400 - 500 nm) is strongly perceived by cryptochromes and other flavin-containing receptors and plays a key role in the regulation of the circadian clock in photosynthetic organisms (Fortunato et al. 2015). Thus, ALAN with strong emission of blue light might be more likely to disrupt circadian regulation and processes regulated by the circadian rhythm.

1.6. Research gaps, aims and structure of the thesis

Despite the wide presence or artificial light at night in the environment, its effects on the ecosystems in artificially illuminated waters are not well understood and its potential impacts on primary producers are rarely considered. Light is a fundamental resource for primary producers and its role in driving and controlling their physiological processes and shaping their communities in aquatic systems have been extensively studied, both under laboratory

conditions and in the field and at the level of single cells, individual organisms or whole communities. Experimental designs for these studies commonly include manipulations of light intensity, length of light and dark phases or exposure to constant light of moderate to high intensities. However, a light regime typical for light-polluted urban and sub-urban environments, a high-level of (broad-emission) light during the day and a low-level of (limited waveband emission) light at night, has rarely been simulated. The first study on primary producers that simulated light regime of a light-polluted waterbody in the laboratory was performed on cyanobacterial cultures of *Microcystis aeruginosa* (Poulin et al. 2014). ALAN at an intensity comparable to that of near-shore urban areas (0.08 µmol m^{-2} s^{-1}, approx. 6.6 lux) was found to significantly affect several photophysiological processes, e.g. increased intracellular concentrations of chlorophyll *a* and chlorophyll *a* not bound to the photosystems, the functional absorption cross-section of photosystem II and maximum quantum yield of charge separation. These changes indicated ALAN decreased photosynthetic efficiency, but no significant effects on growth rates were observed. Whether these effects could be significant in more complex real-world context remained yet to be determined. Hölker et al. (2015) studied the effects of ALAN on mixed microbial communities of autotrophs and heterotrophs from sediments, on a community level. In this study, sediments were exposed to ALAN in the field (6.8 – 8.5 lux, approx. 0.09 µmol m^{-2} s^{-1}) over one year. Both ALAN-illuminated sediments and those that experienced natural light regime in the field were transferred to the laboratory where respiration of microbial communities were measured under mimicked light-pollution conditions (71 lux, approx. 1.3 µmol m^{-2} s^{-1}) and and community composition was assessed. ALAN increased relative abundance of primary producers including diatoms and cyanobacteria, and was indicated to stimulate nocturnal photosynthesis (Hölker et al. 2015). These studies suggested that artificial nocturnal illumination can have significant effects on physiology and community composition of primary producers. However, the light levels applied in the laboratory incubations by Hölker et al. (2015) were relatively high for light-polluted areas, comparable to directly illuminated areas close to the light sources. Thus it remained unclear whether effects of ALAN would also be significant under lower light intensities that are more commonly found in urban and sub-urban waters, e.g. those in the range between the two studied levels (6.6 to 71 lux); and whether a decrease or an increase in growth could be expected under such light intensities. In addition, effects of ALAN were confounded by the light source type, as Poulin et al. (2014) applied high-pressure sodium light (HPS, 6.6 lux) in the laboratory, while Hölker et al. (2015) applied HPS (6.8 – 8.5 lux) illumination in the field, but LED (71 lux) in the laboratory. Different spectral composition of the two lamps may drive commu-

nity changes in different ways and the role of the light quality in effects of artificial nocturnal illumination remained unclear.

This thesis aims to investigate effects of artificial nocturnal illumination on biomass and community composition of freshwater periphyton, with a focus on benthic primary producers. The majority of information available in the literature regarding light and photosynthetic processes describes effects of light levels above the photosynthetic compensation point and surprisingly little information is available about physiological processes that occur under lower light intensities. This clear lack of knowledge about mechanisms through which low levels of ALAN might affect primary producers made it difficult for us to develop a precise hypothesis regarding the anticipated changes in periphyton communities. I chose a light intensity from a range commonly found in light-polluted areas, 20 lux (aprox. 0.31 µmol m^{-2} s^{-1}) (Hale et al. 2013) and intermediate between the two previously studied light levels, 6.6 to 71 lux (Hölker et al. 2015; Poulin et al. 2014). I expected that this intensity would provide enough energy to stimulate nocturnal photosynthesis and net carbon fixation and result in higher biomass of periphyton. In particular, I expected that ALAN would stimulate the growth of taxa well adapted to utilize low-light levels such as diatoms, increasing their proportion in the community. The differential sensitivity and growth of individual taxa in periphyton would additionally be reflected in an altered taxonomic composition under ALAN. I investigated the two currently most commonly used light types for outdoor lighting, high-pressure sodium (HPS) and light-emitting diodes (LED) lights, characterized by distinctly different spectral composition, in particular the amount of blue light. I expected that pronounced emission of blue light emitted by wideband LEDs would have stronger biological impacts on photosynthesis and circadian regulation, compared to the yellow light of HPS. Furthermore, I expected that the complexity and structure of periphyton matrix determined by its colonization stage would play a role in sensitivity of periphyton to ALAN, where increased periphyton complexity of late colonization stages would decrease the sensitivity to ALAN.

I performed manipulative field studies in two contrasting aquatic systems to simulate the night-time light conditions of a light-polluted waterbody and compared biomass and community composition of periphyton grown under ALAN with periphyton grown under a natural light regime. Effects were assessed for the two most commonly used light sources for outdoor lighting and the studies were performed in different seasons to evaluate potentially different responses due to seasonally-driven variation in periphyton community composition and environmental variables. In addition, I assessed the sensitivity of periphyton to ALAN exposure at two different colonization stages.

Chapters 2, 3 and 4 describe my first experimental study, where I simulated light conditions of a light-polluted area using a stream-side flume system on a sub-alpine stream. Nocturnal illumination was supplied with warm-white LED lights and the applied light intensity was 20 lux at the water surface. Experiment was performed in spring and autumn.

In **Chapter 2** I investigated the effects of ALAN on biomass and major group composition (i.e. diatoms, green algae and cyanobacteria) of stream periphyton and assessed the sensitivity of periphyton to ALAN exposure in two different colonization stages, early (one to three weeks of growth) and later (three to six weeks of growth). Here I applied a relatively new method for rapid assessment of periphyton biomass and community composition, the BenthoTorch *in situ* fluorometer.

In **Chapter 3** I applied a more conventional and common method for assessment of mixed algal assemblages, high-performance liquid chromatography, to investigate community composition of ALAN-lit and control stream periphyton of later colonization stages (three to six weeks) and infer potential ALAN-induced changes on the community level from the pigment composition.

In **Chapter 4** I further investigated effects of LED nocturnal illumination on later colonization stages (three to six weeks) of stream periphyton by using DNA metabarcoding for analysis of taxonomic composition, diversity and abundance of algal taxa, in particular diatoms, in the periphyton.

In **Chapter 5** I describe my second experimental study, where I simulated light conditions of a light-polluted area in a shallow, lowland agricultural ditch. I assessed the effects of artificial nocturnal illumination (exposure from two to 13 weeks) on biomass and community composition of periphyton and used pigment composition to infer ALAN_induced community changes. Here I compared effects of two types of light sources, HPS and warm-white LED, using comparable light intensities of around 20 lux at the water surface.

In **Chapter 6** I placed the key findings from the previous chapters in a broader context. I commented on the suitability of the three applied techniques, *in situ* fluorometry, pigment analysis by high-performance liquid chromatography and ordination and the analysis of taxonomic composition by DNA metabarcoding and community similarity indices, to detect community changes induced by artificial nocturnal illumination on periphyton biomass and composition. I discussed the implications of the findings for policy and regulation of artificial illumination of freshwater ecosystems and the possibilities to mitigate its impacts. Finally, I identified knowledge gaps and suggest next steps for further research.

References (Chapter 1)

Aube, M., J. Roby, and M. Kocifaj. 2013. Evaluating potential spectral impacts of various artificial lights on melatonin suppression, photosynthesis, and star visibility. Plos One **8:** e67798.

Biggs, B. J. F. 1996. Patterns in benthic algae of streams, p. 31-56. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Bishop, J. E. 1969. Light control of aquatic insect activity and drift. Ecology **50:** 371-380.

Boston, H. L., and W. R. Hill. 1991. Photosynthesis light relations of stream periphyton communities. Limnol. Oceanogr. **36:** 644-656.

Brand, L. E., and R. R. L. Guillard. 1981. The effects of continuous light and light intensity on the reproduction rates of twenty-two species of marine phytoplankton. J. Exp. Mar. Biol. Ecol. **50:** 119-132.

Bünning, E., and I. Moser. 1969. Interference of moonlight with the photoperiodic measurement of time by plants, and their adaptive reaction. Proc. Natl. Acad. Sci. USA **62:** 1018-1022.

Cashman, M. J., J. D. Wehr, and K. Truhn. 2013. Elevated light and nutrients alter the nutritional quality of stream periphyton. Freshwat. Biol. **58:** 1447–1457.

Ceola, S., F. Laio, and A. Montanari. 2015. Human-impacted waters: New perspectives from global high-resolution monitoring. Water Resour. Res. **51:** 7064-7079.

Devlin, P. F., and S. A. Kay. 2001. Circadian photoreception. Annu. Rev. Physiol. **63:** 677–694.

Dodds, W. K., B. J. F. Biggs, and R. L. Lowe. 1999. Photosynthesis-irradiance patterns in benthic microalgae: Variations as a function of assemblage thickness and community structure. J. Phycol. **35:** 42-53.

Elliott, J. M. 1968. The daily activity patterns of mayfly nymphs (Ephemeroptera). J. Zool. **155:** 201-221.

Falchi, F. and others 2016. The new World Atlas of artificial night sky brightness. Sci. Adv. **2:** e1600377.

Falkowski, P. G., and J. LaRoche. 1991. Acclimation to spectral irradiance in algae. J. Phycol. **27:** 8-14.

Fortunato, A. E., R. Annunziata, M. Jaubert, J.-P. Bouly, and A. Falciatore. 2015. Dealing with light: The widespread and multitasking cryptochrome/photolyase family in photosynthetic organisms. J. Plant Physiol. **172:** 42-54.

Gaston, K. J., J. Bennie, T. W. Davies, and J. Hopkins. 2013. The ecological impacts of nighttime light pollution: A mechanistic appraisal. Biol. Rev. **88:** 912-927.

Gaston, K. J., J. P. Duffy, S. Gaston, J. Bennie, and T. W. Davies. 2014. Human alteration of natural light cycles: Causes and ecological consequences. Oecologia **176:** 917–931.

Gaston, K. J., M. E. Visser, and F. Hölker. 2015. The biological impacts of artificial light at night: The research challenge. Phil. Trans. Roy. Sci. B, **370:** 20140133.

Guo, F., M. J. Kainz, F. Sheldon, and S. E. Bunn. 2016. The importance of high-quality algal food sources in stream food webs – current status and future perspectives. Freshwat. Biol. **61:** 815–831.

Hale, J. D., G. Davies, A. J. Fairbrass, T. J. Matthews, C. D. F. Rogers, and J. P. Sadler. 2013. Mapping lightscapes: Spatial patterning of artificial lighting in an urban landscape. Plos One **8:** e61460.

Hegemann, P., M. Fuhrmann, and S. Kateriya. 2001. Algal sensory photoreceptors. J. Phycol. **37:** 668–676.

Hill, W. R. 1996. Effects of light, p. 121-149. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Hölker, F. and others 2010a. The dark side of light: A transdisciplinary research agenda for light pollution policy. Ecol. Soc. **15:** 13.

Hölker, F., C. Wolter, E. K. Perkin, and K. Tockner. 2010b. Light pollution as a biodiversity threat. Trends Ecol. Evol. **25:** 681-682.

Hölker, F., C. Wurzbacher, C. Weissenborn, M. T. Monaghan, S. I. J. Holzhauer, and K. Premke. 2015. Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. Phil. Trans. Roy. Sci. B. **370:** 20140130.

Hudon, C., and E. Bourget. 1983. The effect of light on the vertical structure of epibenthic diatom communities. Bot. Mar. **26:** 317-330.

Kirk, J. T. O. 1994. Light and photosynthesis in aquatic ecosystems. Cambridge University Press.

Kummu, M., H. d. Moel, P. J. Ward, and O. Varis. 2011. How close do we live to water? A global analysis of population distance to freshwater bodies. Plos One **6:** e20578.

Kyba, C. C. M., and F. Hölker. 2013. Do artificially illuminated skies affect biodiversity in nocturnal landscapes? Landscape Ecol.**:** 1637–1640.

Lange, K., A. Liess, J. J. Piggott, C. R. Townsend, and C. D. Matthaei. 2011. Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. Freshwat. Biol. **56:** 264-278.

Laviale, M., J. Prygiel, Y. Lemoine, A. Courseaux, and A. Creach. 2009. Stream periphyton photoacclimation response in field conditions: Effect of community development and seasonal changes. J. Phycol. **45:** 1072-1082.

Law, R. J. 2011. A review of the function and uses of, and factors affecting, stream phytobenthos. Freshw. Rev. **4:** 135-166.

Lowe, R. L., and Y. Pan. 1996. Benthic algal communities as biological monitors, p. 705-739. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Navara, K. J., and R. J. Nelson. 2007. The dark side of light at night: Physiological, epidemiological, and ecological consequences. J. Pineal Res. **43:** 215-224.

Perkin, E. K., F. Hölker, J. S. Richardson, J. P. Sadler, C. Wolter, and K. Tockner. 2011. The influence of artificial light on stream and riparian ecosystems: Questions, challenges, and perspectives. Ecosphere **2:** 1-16.

Petroutsos, D. and others 2016. A blue-light photoreceptor mediates the feedback regulation of photosynthesis. Nature **537:** 563–566.

Poulin, C., F. Bruyant, M.-H. Laprise, A. M. Cockshutt, J. M.-R. Vandenhecke, and Y. Huot. 2014. The impact of light pollution on diel changes in the photophysiology of *Microcystis aeruginosa*. J. Plankton Res. **36:** 286-291.

Raven, J. A., and C. S. Cockell. 2006. Influence on photosynthesis of starlight, moonlight, planetlight, and light pollution (reflections on photosynthetically active radiation in the universe). Astrobiology **6:** 668-675.

Rich, C., and T. Longcore. 2006. Ecological consequences of artificial night lighting. Island Press.

Richardson, K., J. Beardall, and J. A. Raven. 1983. Adaptation of unicellular algae to irradiance: An analysis of strategies. New Phytol. **93:** 157-191.

Rockwell, N. C. and others 2014. Eukaryotic algal phytochromes span the visible spectrum. Proc. Natl. Acad. Sci. USA **111:** 3871-3876.

Schad, W. 1999. Lunar influence on plants. Earth, Moon, and Planets. An International Journal of Solar System Science **85:** 405–409.

Smol, J. P., and E. F. Stoermer. 2010. The Diatoms: Applications for the environmental and earth sciences. Cambridge University Press.

Steinmann, A. D. 1999. Effects of grazers on freshwater benthic algae. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Aquatic ecology series. Academic Press.

Stevenson, R. J. 1996. An introduction to algal ecology in freshwater benthic habitats. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Wu, Y. 2017. Periphyton: Functions and application in environmental remediation. Elsevier.

Yeh, N. C., and J. P. Chung. 2009. High-brightness LEDs - Energy efficient lighting sources and their potential in indoor plant cultivation. Renewable & Sustainable Energy Reviews **13:** 2175-2180.

2. Artificial light at night decreases biomass and alters community composition of benthic primary producers in a sub-alpine stream

Maja Grubisic[1,2], Gabriel Singer[1], M. Cristina Bruno[3], Roy H.A. van Grunsven[1], Alessandro Manfrin[1,2], Michael T. Monaghan[1], Franz Hölker[1]

[1] *Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany*
[2] *Institute of Biology, Freie Universität Berlin, Berlin, Germany*
[3] *Sustainable Agro-ecosystems and Bioresources Department, IASMA Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy*

Article published as:

Grubisic M., Singer G., Bruno M.C., van Grunsven R.H.A., Manfrin A., Monaghan M.T., and F. Hölker. 2017. Artificial light at night decreases biomass and alters community composition of benthic primary producers in a sub-alpine stream. Limnol. Oceanogr.

http://dx.doi.org/10.1002/lno.10607

2.1. Abstract

Artificial light at night (ALAN) is recognized as a contributor to environmental change and a biodiversity threat on a global scale. Despite its widespread use and numerous potential ecological effects, few studies have investigated the impacts on aquatic ecosystems and primary producers. Light is a source of energy for photosynthesis and a source of information for benthic autotrophs that form the basis of food webs in clear, shallow waters. Artificial night-time illumination thus may affect biomass and community composition of primary producers, leading to bottom-up changes that can cascade throughout the ecosystem. We experimentally mimicked the night-time light conditions of a light-polluted area (approx. 20 lux) in streamside flumes on a sub-alpine stream. We compared the biomass and community composition of periphyton grown under ALAN with periphyton grown under a natural light regime in two seasons using communities in early (up to three weeks) and later (four to six weeks) developmental stages. In early periphyton, ALAN led to a decrease of autotroph biomass in both spring (57% after three weeks) and autumn (43% after two weeks). ALAN decreased the proportion of cyanobacteria in spring by 54% and altered the proportion of the dominant taxon, diatoms, in autumn (11% decrease after two weeks and 5% increase after three weeks of treatment). No significant effects of ALAN were observed for periphyton at later developmental stages. Further work is needed to test whether streams with frequent physical disturbances that reset the successional development of periphyton are more affected by ALAN than streams with more stable conditions. As periphyton is a fundamental component of stream ecosystems, the impact of ALAN might propagate to critical ecosystem functions.

2.2. Introduction

Light pollution that results from the extensive use of artificial light at night (ALAN) is a global phenomenon and one of the fastest-spreading environmental alterations induced by humans (Hölker et al. 2010a; Falchi et al. 2016). ALAN can have several effects on the natural environment (Longcore and Rich 2004; Hölker et al. 2010b). Studies of ecological effects of ALAN have largely focused on terrestrial habitats, while the interest in aquatic systems is relatively recent (Perkin et al. 2014a; Brüning et al. 2015; Hölker et al. 2015; Honnen et al. 2016) despite the fact that freshwaters are often exposed to ALAN from adjacent urban and sub-urban areas (Ceola et al. 2015). Effects are commonly examined at the level of individual species (Gaston et al. 2015) while fewer studies address higher ecological levels such as

communities or ecosystem functions (e.g. Davies et al. 2012; Becker et al. 2013; Meyer and Sullivan 2013).

Light serves as a source of both energy and environmental information for primary producers (Hegemann et al. 2001). The intensity, spectral quality, timing and duration of light all affect photosynthesis and growth of aquatic primary producers as well as their biochemistry and community composition (Richardson et al. 1983; Falkowski and Laroche 1991; Khoeyi et al. 2012). Where light reaches benthic environments, it supports growth of benthic primary producers that develop embedded in a polysaccharide matrix in close association with bacteria and fungi, forming complex periphyton communities (Wetzel 2001). In clear shallow waters, periphyton often forms the base of the food web (Stevenson 1996). Benthic autotrophs are the main components of periphyton and dominate primary production of small and mid-sized streams (Dodds et al. 1999). Periphyton is therefore a food resource for primary consumers in streams and plays a key role in nutrient and carbon cycling (Stevenson 1996; Law 2011). It is sensitive to alterations of physical, chemical and biological environmental conditions, which is why it is commonly used in biological monitoring (Lowe and Pan 1996).

As a result of human population growth and increased urbanization, previously ALAN-naïve freshwater environments are increasingly exposed to artificial illumination at night. Nocturnal artificial light can stimulate photosynthesis (Aube et al. 2013); however it is unclear whether the light levels typically found in ALAN-illuminated aquatic environments, which are of low intensity in comparison to sunlight, produce measurable and relevant effects on their biomass and community composition. Poulin et al. (2014) found that ALAN at a light level of 0.08 µmol m^{-2} s^{-1} (approximately 6.6 lux, as low as 0.004 - 0.08% of natural mid-day irradiance) affected the physiology in unicellular cyanobacteria in laboratory cultures, although no effects on growth were observed. Hölker et al. (2015) found an increase in the abundance of photoautotrophs (diatoms, cyanobacteria) in sediments after five months of exposure to ALAN of approximately 0.09 µmol m^{-2} s^{-1} (6.8 – 8.5 lux). Periphyton is composed of several groups of autotrophs that all differ in minimum light requirements for growth and photosynthesis. Cyanobacteria and diatoms generally grow better at lower light intensities compared to green algae (Richardson et al. 1983) and therefore might benefit from low-light typically supplied by ALAN. Different light regimes may therefore cause differential responses among taxa, resulting in shifts of competitive equilibria and changes in periphyton community composition (Litchman 1998).

Natural light/dark cycles detected by photoreceptors provide information for the regulation of several physiological processes (Kianianmomeni and Hallmann 2014). As one of the most regular and predictable environmental fluctuations, light/dark cycles drive rhythmic changes in biological processes such as synthesis of various cellular components, DNA repair, growth and development in many organisms, often through a circadian clock (Brand and Guillard 1981; Fortunato et al. 2015 and references therein). In majority of photosynthetic organisms, the regulation of a circadian clock is mediated by cryptochromes and other flavin blue-light receptors (Fortunato et al. 2015). Algae can detect light as low as moonlight (approx. 0.1 lux) (Bünning and Moser 1969) and cyanobacteria are also documented to detect and respond to changes in light intensity and spectral quality (Mullineaux 2001). Therefore the disruption of natural light/dark cycles by ALAN may cause dysfunction in circadian rhythms and thereby light-driven physiological processes. If sensitivities differ among taxa, also an altered light/dark regime could represent a selection pressure potentially altering community composition.

In stable habitats dominated by periphyton, e.g. streams with stable flows, the development of periphyton communities follows characteristic patterns of colonization and succession. Biomass accrual over time leads to the establishment of three-dimensional, spatially complex biomass matrices (Biggs 1996) and a shift in growth forms from the dominance of small, adnate diatoms toward higher abundance of erect, stalked forms and finally to filamentous forms of green algae and cyanobacteria (Hudon and Bourget 1983; Biggs 1996). Physical disturbances such as fluctuations in flow associated with discharge peaks or increased wave action, or sediment transport caused by floods and storms, can erode periphyton biomass and thus alter or reset its successional state. These successional patterns are accompanied by increased vertical sub-structuring and differentiation of a periphyton matrix. The development of this microscale architecture is strongly influenced by light conditions and modifies the environmental conditions within the matrix itself, modulating a community response to light (Boston and Hill 1991). Moreover, seasonal variation in environmental conditions causes strong seasonal differences in periphyton community composition (Biggs 1996). In temperate latitudes, variation in light regime is one of the major drivers of seasonal patterns in species composition and autotrophs are in general better adapted to lower light conditions in winter and spring and higher light conditions in summer and autumn (Kirk 1994; Laviale et al. 2009). Thus it is likely that the sensitivity of periphyton to ALAN will vary across seasons as well.

We used streamside artificial flumes fed by a sub-alpine stream to investigate the effects of night-time illumination on periphyton. We mimicked the conditions of light-polluted areas of urban and sub-urban streams and measured its effects on biomass and community composition of periphyton in early ("developing") and late ("pre-established") developmental stages. We conducted the experiment in two seasons (spring and autumn) to account for seasonal differences in community composition and (non-ALAN associated) environmental conditions. We hypothesized that ALAN would stimulate photosynthesis, resulting in higher biomass of periphyton. In particular, we expected ALAN to stimulate the growth of diatoms in periphyton, thereby altering periphyton community composition. We also expected the effects of ALAN to depend on the periphyton developmental stage, with later stages of spatially complex communities being less sensitive to ALAN.

2.3. Methods

2.3.1. Study site and experimental design

Experiments were conducted in a set of five metal flumes situated in the riparian zone of the Fersina stream in Trentino Province, Northeastern Italy (46° 04′ 32″ N, 11° 16′ 24″ E) at 577 m asl in spring and autumn 2014. The Fersina is a 2nd order snowmelt-fed gravel-bed stream originating at an altitude of 2005 m. It is approximately 14 km long, with a 171 km^2 watershed receiving the contribution of numerous small streams that descend from lateral valleys. The streamside flumes on the Fersina have been used for ecohydrological studies on periphyton (Cashman et al. 2016) and benthic macroinvertebrates (Carolli et al. 2012; Bruno et al. 2013; 2016). The flume system (Fig. 3) is located on the right bank, with no history of direct exposure to ALAN in the entire upstream section. It consists of five metal, U-shaped flumes that are 20 m long and 30 cm wide with either 30 cm (flumes A-C) or 50 cm (flumes D, E) high side walls. Flumes are directly fed by water that is diverted from the stream through a loading tank equipped with a sluice gate for discharge regulation. A metal mesh (3x5 cm opening) prevents large material and fish from entering the flumes while allowing the colonization by periphyton and macroinvertebrate fauna. A baseflow of 0.05 m^3 s^{-1} and velocity of 0.4 m s^{-1} was established by manipulating a sluice gate in all flumes 6 months before starting the experiment and kept constant throughout the experimental period. The flume bottom was covered with a 20 cm thick layer of cobbles of approximately 10 cm diameter and a layer of gravel and sand deposited by the water flow.

Figure 3. Side view of the stream-side experimental flume system on the Fersina stream (Trentino Province, NE Italy). Flow runs from left to right. Photo: M. Grubisic.

On March 4 (for spring sampling) and September 1 (for autumn sampling) we evenly distributed 16 white unglazed ceramic tiles (9.8 cm x 19.6 cm) into each flume along its entire length. The tiles were used as substrate for the development of periphyton. Each was placed on top of the cobble layer, centrally in the flumes at a maximum water depth of 5 cm. We left the tiles for 26 days in spring and 22 days in autumn in order to facilitate the natural development of a "pre-established" community prior to the beginning of the experimental treatment (Oemke and Burton 1986 and references therein). The growth time in September was shorter as the higher water temperature stimulated periphyton growth.

On March 31 and September 24, artificial light was installed by mounting battery-powered warm-white LED strips (12 V, Barthelme, Nürnberg, Germany; 3000 K color temperature measured with spectroradiometer specbos 1211UV, JETI, Jena, Germany; Fig. 4) on wires either above the upstream or the downstream section of each flume (chosen randomly). This experimental setup resulted in a design with a total of five lit sections and five control sections in 10 flume sections of 10 m length. Lightproof plastic foil curtains were hung on steel wires between half-flume sections and longitudinally between the flumes, to prevent the LED light from spreading into the control sections, which were exposed to natural light/dark regime. Curtains were removed during the day to allow direct sunlight to reach all flume sections. The light levels were measured below the water surface with an ILT1700 underwater

photometer (International Light Technologies Inc., Peabody, Massachusetts, USA) after astronomical twilight on the nights of the new moon, on March 30 and September 23 (Table 1). Mean illumination in the lit sections amounted to 20.3 ± 1.8 lux (mean and SD, n = 20; approx. 0.31 µmol m^{-2} s^{-1}), a light level comparable to those found in urban environments (Hale et al. 2013).

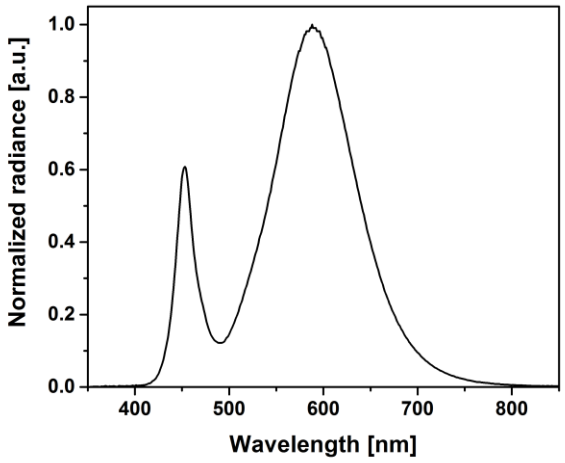

Figure 4. Spectral composition of LED lights used in the study (12 V, 3000 K, Barthelme, Nürnberg, Germany).

A timer was used to automatically turn the lights on and off at civil twilight and dawn over a period of three weeks. The length of the illumination period was chosen to cover the full range of natural nocturnal light levels, i.e. from new moon to full moon illumination. A longer illumination period was avoided in order to avoid periphyton reaching senescence phase in succession, which could drive community changes independently from ALAN. We measured flow velocity using a hand-held current meter (Global Water Flow Probe, Global Water Instrumentation, College Station, Texas, USA) and physico-chemical parameters using a WTW© handheld meters for oxygen, pH, conductivity and turbidity (WTW GmbH, Weilheim, Germany) (Table 1; Appendix A Table S1).

Table 1. Environmental parameters averaged over the experimental period for the two investigated seasons (n = 20)

	Spring		Autumn	
	mean	SD	mean	SD
Conductivity (µS cm^{-1})	95.67	12.80	142.70	1.88
Temperature (°C)	6.6	1.3	13.4	0.1
Oxygen (mg L^{-1})	11.59	0.96	8.83	0.11
Oxygen (%)	101.2	6.0	90.1	1.3
pH	7.7	0.8	8.1	0.1
Turbidity (NTU)	1.53	0.34	0.39	0.17
Velocity (m s^{-1})	0.37	0.18	0.34	0.11
Light at night in D sections (lux)	0.0027	0.0008	0.0012	0.0006

2.3.2. Sampling procedure

On the first sampling day of each growth period (March 31 and September 24), we sampled four tiles from each flume section ("pre-established periphyton") and deployed 12 new, clean tiles evenly along each flume section for the later collection of "developing" periphyton, so that each flume contained a total of 24 tiles. From this point onwards we sampled four replicate tiles with periphyton of identical developmental stage from each flume section on a weekly basis for three weeks (Table 2). This allowed us to analyze pre-established periphyton using a replicated Before-After-Control-Impact (BACI) design, while developing periphyton was analyzed as a time series because all tiles were uncolonized at the start of the experiments.

Table 2. Overview of tile manipulations and sampling dates in two experimental seasons

	Season	Deployed	No. of weeks prior to the treatment	Pre-treatment sampling	Lights turned on	During –treatment sampling			End of treatment sampling	No. of weeks of exp. treatment for each collected tile
Pre-established periphyton	Spring	4-Mar	4	31-Mar	31-Mar	-	-	-	23-Apr	3
	Autumn	1-Sep	3	24-Sep	24-Sep	-	-	-	16-Oct	3
Developing periphyton	Spring	31-Mar	-	-	31-Mar	7-Apr	14-Apr	21-Apr	-	1, 2, 3
	Autumn	24-Sep	-	-	24-Sep	1-Oct	8-Oct	14-Oct	-	1, 2, 3

Tiles were carefully removed from the flumes to minimize biomass loss due to sloughing. Any non-periphytic material (e.g. Simulidae larvae) attached to the sides and the bottom was removed with forceps. Each tile was placed into a plastic box (23 x 14 x 6.5 cm) and carefully covered with pre-filtered (GF/F glass-fiber filter, 0.7 µm nominal pore size, Whatman Ltd., Maidstone, UK) water from the flumes. We measured periphyton biomass in the field using an in-situ deployable fluorometer (BenthoTorch, bbe Moldaenke GmbH, Schwentinental, Germany). This instrument is designed for rapid quantification of biomass of benthic autotrophs based on in vivo chlorophyll *a* fluorescence at 690 nm and for assessment of community composition by discrimination of diatoms, green algae and cyanobacteria based on the fluorescence of marker pigments with fluorescent signatures at 470, 525 and 610 nm (Harris and Graham 2015). Studies that examined the accuracy and sensitivity of the BenthoTorch (BT) report discrepancies in relative percentage of different groups compared to results obtained by standard laboratory procedures such as ethanol extraction of chl *a*, spectrophotometric determination of chl *a*, or analysis of biovolume with a microscope (Kahlert and Mckie 2014; Harris and Graham 2015). The accuracy of BT measurements was also found to decline with BT chl *a* concentrations > 4 µg cm^{-2}. We took eight 1-cm^2 BT measurements of undisturbed periphyton for each tile, distributed across the tile surface. All measurements were performed in the morning (8:00 to 12:00). The periphyton was then scraped from each sampled tile with a razor and a tooth brush and the tile was rinsed with pre-filtered flume water. The resulting periphyton suspension was collected into a 250 mL plastic bottle, labelled and stored on ice pending analysis in the laboratory within 24 hours.

2.3.3. Laboratory procedures

The total volume of the periphyton suspension was determined with a measuring cylinder. After vigorous shaking, aliquots for determination of dry mass were concentrated on pre-combusted, pre-weighed 25 mm Whatman GF/F glass-fiber filters by vacuum filtration, dried at 65°C until constant weight and re-weighed. Additional aliquots for pigment analysis were concentrated on filters and stored in 2 mL safety reaction vessels. These filters were transferred to -80°C for a minimum of 48 hours to stimulate cell lysis and subsequently freeze-dried and stored at -20°C pending analysis by high-performance liquid chromatography (HPLC) (Waters, Millford, MA, U.S.A.). Pigments were analyzed only for pre-established periphyton, following the procedure described in Woitke et al. (1994) and Shatwell et al. (2012). Pigments were identified and quantified by their retention time and absorp-

tion spectra from standards and 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. Chl *b*, chl *c* and fucoxanthin were determined from the absorption readings at 440 nm.

2.3.4. Data analysis

We used Pearson's correlation analyses to compare the measurements obtained by the BT with those based on HPLC-derived data and the measurements of dry mass (DM). Spring and autumn sets of samples were analyzed separately, as there are seasonal differences in the periphyton community composition. To test for effects of ALAN on total biomass (log-transformed), absolute (log-transformed) and relative biomass of the major groups (diatoms, green algae and cyanobacteria as distinguished by the BT) we used linear mixed-effects models (LMM) (Zuur et al. 2009) as available in the nlme package (Pinheiro et al. 2015) for R (Version 3.1.3., R Core Team 2015). We included treatment ("lit" and "control") and time ("before" and "after" for pre-established periphyton, "2 weeks" and "3 weeks" for developing periphyton) as fixed factors in the model, while flume and tile were defined as nested random factors to avoid pseudoreplication and account for spatial dependency between replicate tiles and sections within the individual flumes. When the observed variance differed between the levels of fixed factors (treatment or time), these were used as variance covariates (Zuur et al. 2009). The same model was used to test if ALAN affected the chl *a*:DM ratio. This is a commonly used indicator for the proportion of autotrophic biomass in the periphyton community (Stevenson 1996) and is related to physiological acclimation of periphyton to light conditions, as intracellular concentrations of photosynthetic pigments increase in adaptation to low light intensities (Falkowski and Laroche 1991). Changes in pigment ratios may indicate changes in intracellular pigment concentrations or reflect alterations in the community composition (Jeffrey et al. 1997).

For pre-established periphyton, the experimental design followed a replicated BACI approach. Therefore, any effect of ALAN is represented by the interaction term treatment x time. For developing periphyton, the starting phase was the same for all treatments (no periphyton) and the effect of ALAN is considered to be directly represented by the treatment main effect. Pairwise comparisons of significant interactions were performed using the glht function from the multcomp package for R (Hothorn et al. 2008) with Benjamini-Hochberg *p* value adjustments.

2.4. Results

2.4.1. Comparison of BenthoTorch and HPLC biomass measurements

Chlorophyll *a* is a commonly used proxy of autotroph biomass, as it is present in all algae and cyanobacteria. The BT measurements for total biomass of autotrophs in pre-established periphyton were correlated with the concentrations of chl *a* determined by HPLC ($r = 0.93$, $p < 0.01$, Appendix A Fig. S1a). The BT-measured biomass of diatoms was also correlated with the concentrations of their marker pigments (i.e. chl *c*: $r = 0.95$, $p < 0.01$, Fig. S1b; fucoxanthin $r = 0.94$, $p < 0.01$, Fig. S1c). In contrast, the BT-measured biomass of green algae was only weakly correlated with chl *b* ($r = 0.34$, $p < 0.01$, Fig. S1d). Since phycocyanins cannot be identified by our used HPLC protocol, it was not possible to compare the BT-measured biomass of cyanobacteria with HPLC measurements. Both proxies of autotroph biomass, BT-based total biomass of autotrophs and HPLC-based concentration of chl *a*, correlated strongly with the directly measured dry mass of the periphyton, that includes both autotrophs and non-autotrophs (BT total: $r = 0.93$, $p < 0.01$, Fig. S1e; chl *a*: $r = 0.94$, $p < 0.01$; Fig. S1f). In total only 0.7% of periphyton biomass measurements in our experiments were above 4 µg cm^{-2}, the reported upper threshold for unbiased and accurate BT performance (Harris and Graham 2015). Because of the strong correlations observed and the low number of measurements potentially affected by the instrument accuracy, we conclude that the BT provided accurate estimates of autotroph biomass and present only BT-based data hereafter.

2.4.2. Biomass

The biomass of newly developing periphyton was below the detection limit of the BT (0.01 µg cm^{-2}) after one week of growth, but was detectable and measurable after two and three weeks. The total biomass of autotrophs (µg cm^{-2}) increased over time in both lit and control periphyton in both seasons (Fig. 5a,b. LMM, time: $F_{1,70} = 99.38$, $p < 0.0001$ in spring, time: $F_{1,72} = 18.79$, $p < 0.0001$ in autumn). In spring, significant interaction was found between treatment and time (LMM: treatment x time $F_{1,70} = 8.56$, $p = 0.005$). Pairwise comparisons indicated that the biomass did not differ between lit and control periphyton (Fig. 5a, $p = 0.13$) after two weeks of treatment; but that the biomass of the lit periphyton was significantly lower (57%, based on median values) than the control periphyton (Fig. 5a, $p = 0.008$) after three weeks. In autumn, there were significant effects of treatment (LMM: treatment $F_{1,72} = 4.20$, $p = 0.04$) and no significant interaction with time (LMM: treatment x time $F_{1,70} = 1.99$, $p = 0.16$). After two weeks of treatment the biomass of lit periphyton was 43% lower (median,

Fig. 5b) than of control periphyton (p = 0.01), while after three weeks there were no differences between the two treatments. In pre-established periphyton, the total biomass of autotrophs increased over time in both lit and control periphyton in both seasons (Fig. 5c,d. LMM, time: $F_{1,72}$ = 36.9, p < 0.001 in spring, time: $F_{1,72}$ = 191.7, p < 0.001 in autumn). There was no significant difference in biomass between lit and control pre-established periphyton in either season (LMM, spring: treatment x time $F_{1,72}$ =.0.10, p = 0.76, autumn $F_{1,72}$ =.0.64, p = 0.43).

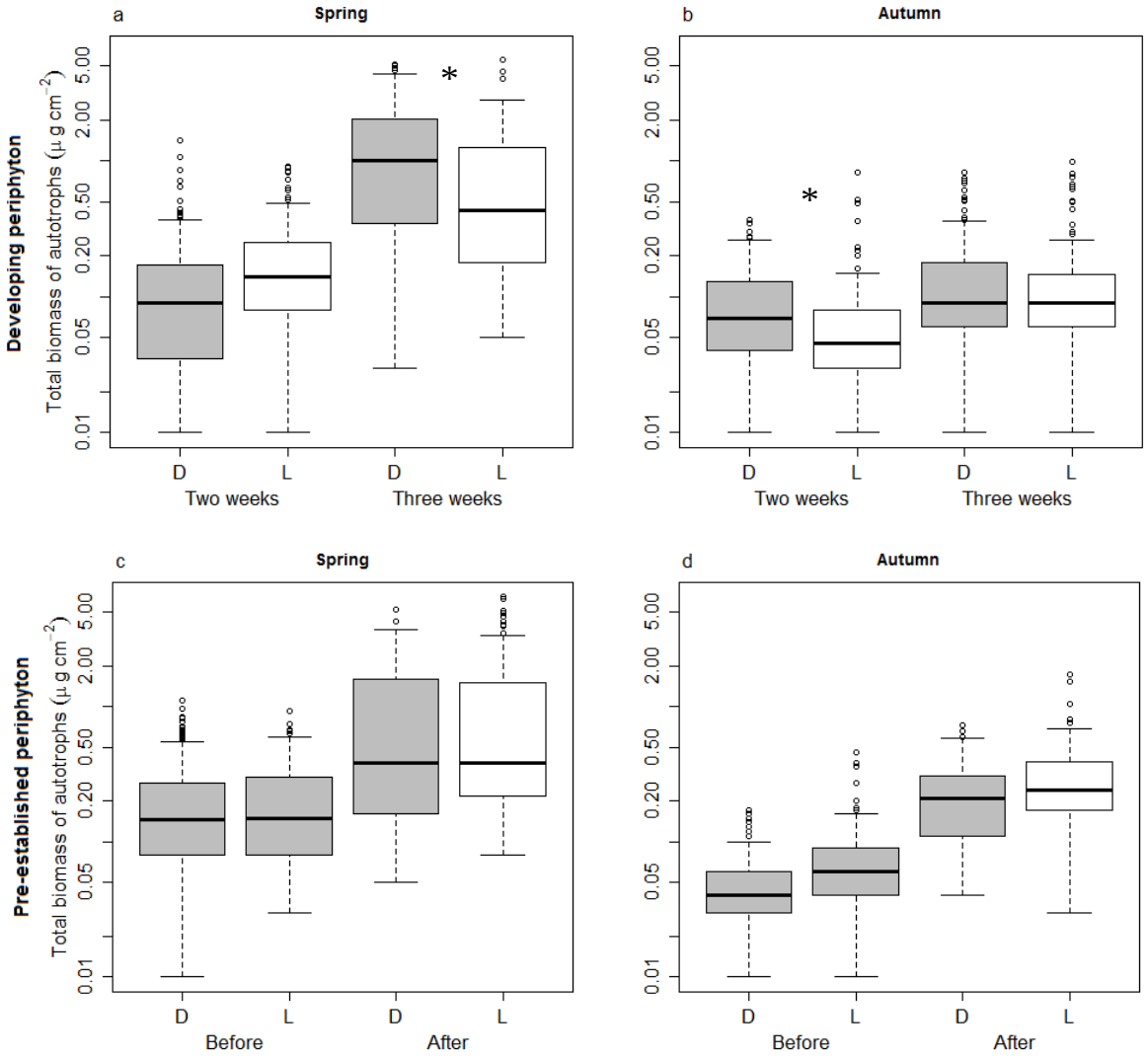

Figure 5. Total biomass of autotrophs (µg cm^{-2}) measured in two experimental seasons with the BenthoTorch (a, b) developing periphyton (single measurements n = 1265); (c, d) pre-established periphyton (single measurements n = 1263) in lit (L) and control (D) flumes sections. Box: median, IQR; whisker: range (5 - 95% values). Data on Log-scale. Asterisk indicates significant difference between the two treatments (p < 0.05, linear mixed models and pairwise comparisons with Benjamini-Hochberg correction).

In pre-established periphyton, the ratio of chl a: DM was not affected by artificial nocturnal illumination in either season, indicating that the proportion of autotrophs in the periphyton community did not change due to different light environment induced by ALAN, nor that periphytic algae responded to ALAN with an increase in the intracellular concentration of photosynthetic pigments.

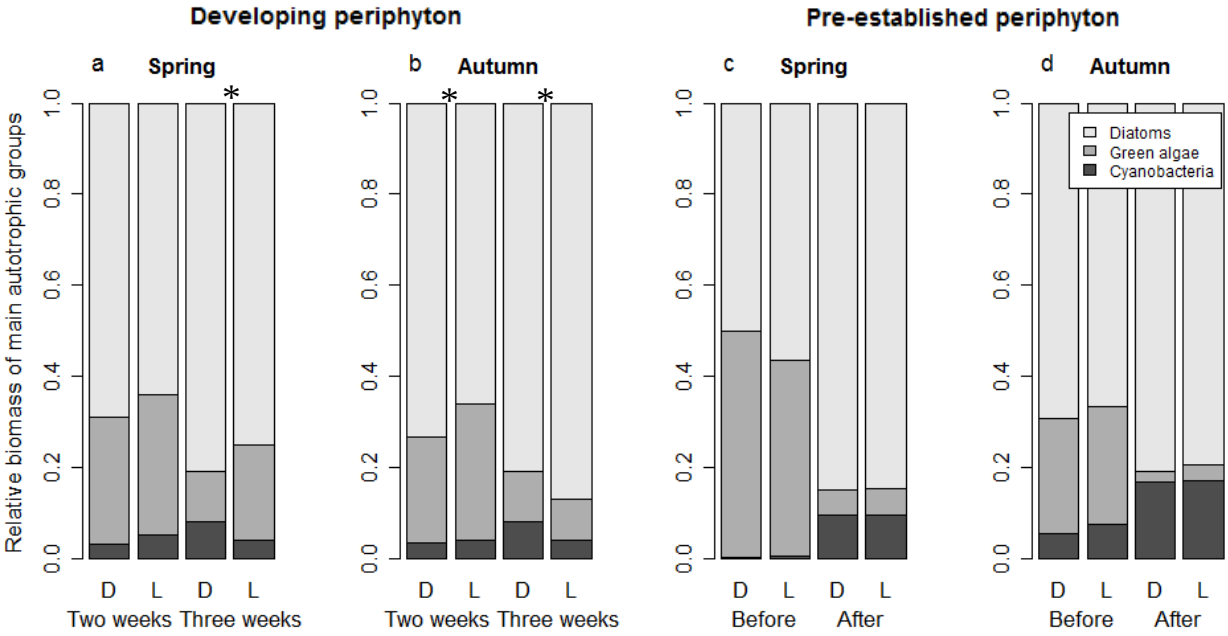

Figure 6. Relative biomass of major autotrophic groups (diatoms, green algae and cyanobacteria) measured in two experimental seasons with the BenthoTorch in: (a, b) developing periphyton (single measurements n = 1265); (c, d) pre-established periphyton (single measurements n = 1263) in lit (L) and control (D) flume sections. Asterisk indicates significant differences in proportions of diatoms or cyanobacteria between the two treatments ($p < 0.05$, linear mixed models and pairwise comparisons with Benjamini-Hochberg correction).

2.4.3. Community composition

Diatoms remained the dominant autotrophs in both lit and control periphyton communities at all times (Fig. 6). Because the biomass of green algae measured with BT did not correlate with their marker pigments identified by HPLC, green algae were not analyzed further. The proportion of diatoms increased with time in both seasons and in both developing (Fig. 6a,b. LMM, spring: time $F_{1,71} = 12.25$, $p < 0.002$, autumn: time $F_{1,73} = 40.21$, $p < 0.001$) and in pre-established communities (Fig. 6c,d. LMM, spring: time $F_{1,72} = 248.18$, $p < 0.001$, au-

tumn: time $F_{1,72} = 0.11$, $p < 0.001$). For developing periphyton there was a significant treatment x time interaction in autumn (Fig. 6b. LMM: treatment x time $F_{1,73} = 9.93$, $p = 0.002$). Pairwise comparisons indicated that lit periphyton had 11% lower (based on median values) proportion of diatoms after two weeks of treatment ($p = 0.01$), but 5% higher proportion after three weeks of treatment ($p = 0.04$) relative to control periphyton. The absolute biomass of diatoms was not altered by the treatment in autumn (Appendix A Table S2, LMM: treatment $F_{1,72} = 3.25$, $p = 0.07$, treatment x time $F_{1,72} = 3.14$, $p = 0.08$). In spring, the proportion of diatoms in developing periphyton did not differ between the lit and control periphyton, although a higher absolute biomass of diatoms was found in lit periphyton compared to the control (LMM: treatment x time $F_{1,70} = 8.93$, $p = 0.004$; pairwise comparisons: lit to control after two weeks $p = 0.18$, after three weeks $p = 0.003$). In the pre-established periphyton, ALAN had no effect on diatoms in either season.

The proportion of cyanobacteria decreased with time in both seasons and in both developing (Fig. 6a,b. LMM, spring: time $F_{1,72} = 42.99$, $p < 0.001$, autumn: time $F_{1,74} = 6.16$, $p = 0.01$) and in pre-established periphyton (Fig. 6c,d. LMM, spring: time $F_{1,74} = 110.74$, $p < 0.001$, autumn: time $F_{1,74} = 33.48$, $p < 0.001$). For developing periphyton there was a significant treatment x time interaction in spring (Fig. 6a. LMM: treatment x time $F_{1,70} = 6.47$, $p = 0.01$). Pairwise comparisons indicated that the proportions did not differ between the lit and the control periphyton after two weeks of treatment ($p = 0.82$), but the proportion of cyanobacteria in lit periphyton was 54% lower than in control (based on median values) after three weeks of treatment ($p = 0.005$). Cyanobacteria decreased by 81% in absolute biomass in lit periphyton in spring (Table S2, LMM: treatment x time $F_{1,70} = 11.47$, $p = 0.001$, pairwise comparisons: lit to control periphyton after two weeks of treatment $p = 0.83$, after three weeks $p < 0.001$), but the values were generally low and below 1 µg cm^{-2} (Table S2). In pre-established periphyton, ALAN did not affect the proportion of cyanobacteria in autumn (LMM: treatment $F_{1,72} = 1.80$, $p = 0.18$, treatment x time $F_{1,72} = 2.00$, $p = 0.16$). In the pre-established periphyton, ALAN had no effects on cyanobacteria in either season.

2.5. Discussion

We found reduced biomass of developing periphyton (up to three weeks old) in the flume sections that experienced night-time illumination in both spring and autumn. The proportions of cyanobacteria and diatoms were also affected by ALAN but in different seasons. In spring, ALAN decreased the proportion of cyanobacteria and in autumn altered the propor-

tion of diatoms, with an initial decrease in lit periphyton after two weeks of treatment, but an increase after three weeks relative to the control. The observed effects of ALAN on periphyton biomass and cyanobacteria were stronger in spring than in autumn, while the opposite was measured for the proportion of diatoms. Seasonal variation in species composition driven by non-ALAN related environmental variables might therefore be important modulators of community response to ALAN. The sensitivity to ALAN depended on the periphyton developmental stage: significant effects were observed in early (up to three weeks) but not in later (four to six weeks) developmental stages. Our results suggest that systems dominated by periphyton in early developmental stages may be more sensitive to ALAN. Therefore, ALAN might reduce resilience of periphyton communities in streams and shoreline habitats subjected to frequent physical perturbations that scour the periphyton biomass and reset periphyton development.

The presence of ALAN creates an environment with alternating phases of high-intensity natural light during the day and low-intensity artificial light during the night. These light conditions have rarely been studied, however there are several aspects that can be discussed and compared to the current literature. By replacing the dark phase in a natural light/dark cycle with low-light illumination, ALAN may provide conditions comparable to those of continuous illumination, with two alternating phases of light intensity. Continuous light can have both positive and negative effects on plants and microalgae for reasons that are still poorly understood (see reviews from Sysoeva et al. 2010; Velez-Ramirez et al. 2011). Many species of algae, plants and lower plants display reduced growth, productivity and photosynthetic efficiency, including reduced quantum yield and lower maximum rates of electron transport and Rubisco carboxylation (Brand and Guillard 1981; Velez-Ramirez et al. 2011 and references therein). The light intensities applied in these studies are of a constant level, usually several orders of magnitude higher than those applied in our experiment and in the range of daylight intensities (75 – 500 µmol m^{-2} s^{-1} compared to approx. 0.31 µmol m^{-2} s^{-1} in our experiment); however some of these effects have been demonstrated to occur at lower light levels as well (Poulin et al. 2014). Maintaining active photosynthesis under low-level ALAN might be energetically costly (Poulin et al. 2014; Hölker et al. 2015). Furthermore, Zevenboom and Mur (1984) reported that the cyanobacteria *Microcystis aeruginosa* required a dark period to obtain maximum growth rate. We observed that also in semi-natural conditions, replacing the dark phase of a natural light/dark cycle with low-light illumination (approx. 20 lux) can reduce the biomass of periphyton.

Many cellular processes such as chloroplast differentiation, DNA repair, cell division, embryogenesis and gametogenesis depend on light/dark cycles (Hegemann et al. 2001) and a dark period might be critical for stress recovery and repair (Gaston et al. 2013). Under continuous light, the clock genes in moss were shown to express arrhythmic profiles (Okada et al. 2009) and this may be also occurring in algae. The disruption of circadian clocks and dependent physiological and developmental processes might therefore explain the observed periphyton biomass decrease in early developmental stages under ALAN in our experiment.

The minimum light intensity that supports growth based on aerobic photosynthesis is considered to be between 0.01 µmol m^{-2} s^{-1} (approx. 0.5 lux) and 0.1 µmol m^{-2} s^{-1} (approx. 7.4 lux), slightly above the maximum light of a full moon on a clear night (0.005 µmol m^{-2} s^{-1}, approx. 0.3 lux) (see discussion in Raven and Cockell 2006 and references therein). These thresholds are far below the ALAN levels applied in our experiment (20 lux) therefore it is likely that nocturnal photosynthesis did occur under ALAN. However, the ALAN treatment did not result in an increase of periphyton biomass. The potential positive effects of ALAN on biomass through nocturnal photosynthesis were likely offset by its negative effects, e.g. through disruption of the circadian clocks or energy costs of maintaining an active photosynthesis, resulting in the neutral or negative effects on periphyton biomass that we observed.

Periphyton growth and biomass are the result of an interaction between species traits available in the community and external factors such as environmental conditions and grazing (Biggs et al. 1998). In our study, non-ALAN associated environmental conditions varied minimally across flumes (Table S1) due to the same inflowing water, short retention time and the controlled flumes setting. The flumes were colonized by macroinvertebrate fauna, including immature stages of Baetidae and to a lesser extent Heptageniidae (A. Manfrin, unpubl.) as predominant grazers/scrapers that feed on epilithic periphyton, algae and detritus. Therefore, the indirect effects of ALAN on periphyton, due to potential changes in grazing activity by macroinvertebrates, cannot be excluded. Both Baetidae and Heptageniidae are common in mountain streams (Hieber et al. 2005) where they hide between the rocks and in crevices during the day and move to forage at night (Bishop 1969). Both taxa are nocturnally photophobic and the number of individuals that move to the upper surface of rocks was shown to decline by 85% under nocturnal illumination of 5 lux (Elliott 1968). Because the tiles were placed on top of the cobble layer, directly exposed to ALAN and did not provide any cover, it is likely that periphyton on the lit tiles was grazed less, or at least not more than in the control sections. If grazers were to have any effect on the periphyton biomass, we would expect less grazing and thus higher periphyton biomass in lit sections relative to controls. However, the lower

biomass we recorded in the lit sections suggests that grazers were not a strong determinant of the periphyton biomass. Assessment of macroinvertebrate communities by Manfrin et al. (unpubl.) confirmed that there were no differences in macroinvertebrate communities between the lit and dark sections and that grazing was not increased under ALAN.

The periphyton was largely composed of diatoms in our experiments. Diatoms are often the dominant group in streams and rivers worldwide (Biggs et al. 1998) and many species are adapted to light-limited conditions, such as heavy shade (Allan and Castillo 2007). The ability to grow under low light levels might provide them with a selective advantage over other groups in the periphyton community under ALAN conditions, as suggested by (Hölker et al. 2015). In contrast to our expectations, the proportion of diatoms only increased in periphyton in early developmental stages (up to three weeks) after three weeks of ALAN treatment in autumn, but a decreased proportion was observed after two weeks. No changes were found for later developmental stages (four to six weeks). Since the periphyton communities significantly changed over time, the observed non-linear response may be related to succession in species composition.

A decrease in cyanobacteria by 54% found for lit periphyton in spring indicated that this group was strongly negatively affected by ALAN in early developmental stages. Similar to other autotrophs, cyanobacteria are able to sense and respond to altered light conditions and possess a circadian rhythm that changes temporal patterns of gene regulation (Mullineaux et al. 2001). The disruption of this circadian regulation by ALAN may be the mechanism behind the observed decrease in early developmental stages.

Seasonal variation in periphyton composition and its physiological adaptation to external light conditions may have reflected in the differential seasonal response of periphyton to ALAN. Periphyton differed in community composition between the seasons (Fig. 6) and may have been composed of different taxa with different light preferences in spring and autumn. Winter and spring communities are known to be better adapted to the low-light levels of the preceding winter (Kirk 1994) and this might have increased the sensitivity of spring periphyton to perceive and respond to low-light levels, compared to the periphyton in autumn.

The fact that effects of ALAN on both biomass and community composition were observed only for developing periphyton supports our hypothesis that periphyton sensitivity to ALAN is higher in early developmental stages compared to later ones. Thicker periphyton biofilms are resistant to high-light stress (Hill 1996) because of the light attenuation and self-shading that occur inside the complex periphyton matrix. This is likely also true for ALAN

and might explain the reduced sensitivity of periphyton in later developmental stages we observed. Seasonally different responses to ALAN we observed might be due to the seasonal variation in community composition detected with BT (Fig. 6) and DNA metabarcoding analysis of 18S-rRNA gene targeting diatoms (Chapter 4), as well as seasonal changes in environmental variables (Table 1).

2.6. Conclusions

The use of artificial light at night is increasing worldwide and therefore the ecological consequences of light pollution are increasing as well (Pawson and Bader 2014). It is known that ALAN affects aquatic microorganisms (Poulin et al. 2014; Hölker et al. 2015), insects (Perkin et al. 2014b; Honnen et al. 2016), and fish (Riley et al. 2012; Brüning et al. 2015). Our study shows that artificial nocturnal illumination, with white LED, can also influence biomass and community composition of aquatic primary producers, the basal food resource for consumers. A better mechanistic understanding of impacts of ALAN is necessary to predict long-term consequences and interactions with other factors such as trophic interactions or anthropogenic stressors such as eutrophication or climate change. Further research on underlying physiological responses, taxonomic sensitivity and the regulation of ecosystem metabolism may give an insight in the non-linear responses of ALAN observed in this experiment. Assessing effects of ALAN generated by different light sources, at different light levels and in different aquatic systems is urgently needed in order to identify and mitigate adverse ecological effects of light pollution.

Acknowledgements

We thank the Servizio Bacini Montani, Autonomous Province of Trento, for building and maintaining the flumes; Stefan Heller (IGB), Lorenzo Forti and Martino Salvaro (Department of Environmental, Civil and Mechanical Engineering of the University of Trento) for their help in setting up the experiments. We thank Cécile Perillon (IGB) and Garabet Kazanjian (IGB) for their support in the laboratory, Barbara Stein (IGB) for performing HPLC analysis, Kate Laskowski (IGB) for advice on statistical analysis, Andreas Jechow (IGB) for advice on light measurements and Figure 2. and Thomas Mehner (IGB) for valuable comments on an early version of the manuscript. We thank four anonymus reviewers for suggestions for improvements on the manuscript. This work has been carried out within the Erasmus Mundus Joint Doctorate Program SMART, funded by the Education, Audiovisual and Culture

Executive Agency of the European Commission. Funding was also provided by the Federal Ministry of Research and Technology, Germany (BMBF-033L038A) and the Federal Agency for Nature Conservation, Germany (3514821700).

References (Chapter 2)

Allan, J. D., and M. M. Castillo. 2007. Stream ecology: Structure and function of running waters. Springer.

Aube, M., J. Roby, and M. Kocifaj. 2013. Evaluating potential spectral impacts of various artificial lights on melatonin suppression, photosynthesis, and star visibility. Plos One **8:** e67798.

Becker, A., A. K. Whitfield, P. D. Cowley, J. Jarnegren, and T. F. Naesje. 2013. Potential effects of artificial light associated with anthropogenic infrastructure on the abundance and foraging behaviour of estuary-associated fishes. J. Appl. Ecol. **50:** 43-50.

Biggs, B. J. F. 1996. Patterns in benthic algae of streams, p. 31-56. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Biggs, B. J. F., R. J. Stevenson, and R. L. Lowe. 1998. A habitat matrix conceptual model for stream peryphyton. Arch. Hydrobiol. **143:** 21-56.

Bishop, J. E. 1969. Light control of aquatic insect activity and drift. Ecology **50:** 371-380.

Boston, H. L., and W. R. Hill. 1991. Photosynthesis light relations of stream periphyton communities. Limnol. Oceanogr. **36:** 644-656.

Brand, L. E., and R. R. L. Guillard. 1981. The effects of continuous light and light intensity on the reproduction rates of twenty-two species of marine phytoplankton. J. Exp. Mar. Biol. Ecol. **50:** 119-132.

Brüning, A., F. Hölker, S. Franke, T. Preuer, and W. Kloas. 2015. Spotlight on fish: Light pollution affects circadian rhythms of European perch but does not cause stress. Sci. Total Environ. **511:** 516-522.

Bruno, M. C., M. J. Cashman, B. Maiolini, S. Biffi, and G. Zolezzi. 2016. Responses of benthic invertebrates to repeated hydropeaking in semi-natural flume simulations. Ecohydrology **9:** 68-82.

Bruno, M. C., A. Siviglia, M. Carolli, and B. Maiolini. 2013. Multiple drift responses of benthic invertebrates to interacting hydropeaking and thermopeaking waves. Ecohydrology **6:** 511-522.

Bünning, E., and I. Moser. 1969. Interference of moonlight with the photoperiodic measurement of time by plants, and their adaptive reaction. Proc. Natl. Acad. Sci. USA **62:** 1018-1022.

Carolli, M., M. C. Bruno, A. Siviglia, and B. Maiolini. 2012. Responses of benthic invertebrates to abrupt changes of temperature in flume simulations. River Res. Appl. **28:** 678-691.

Cashman, M. J., G. L. Harvey, G. Wharton, and M. C. Bruno. 2016. Wood mitigates the effect of hydropeaking scour on periphyton biomass and nutritional quality in semi-natural flume simulations. Aquat. Sci.**:** 1-13.

Ceola, S., F. Laio, and A. Montanari. 2015. Human-impacted waters: New perspectives from global high-resolution monitoring. Water Resour. Res. **51:** 7064-7079.

Davies, T. W., J. Bennie, and K. J. Gaston. 2012. Street lighting changes the composition of invertebrate communities. Biol. Lett. **8:** 764-767.

Dodds, W. K., B. J. F. Biggs, and R. L. Lowe. 1999. Photosynthesis-irradiance patterns in benthic microalgae: Variations as a function of assemblage thickness and community structure. J. Phycol. **35:** 42-53.

Elliott, J. M. 1968. The daily activity patterns of mayfly nymphs (Ephemeroptera). J. Zool. **155:** 201-221.

Falchi, F. and others 2016. The new world atlas of artificial night sky brightness. Sci. Adv. **2:** e1600377.

Falkowski, P. G., and J. Laroche. 1991. Acclimation to spectral irradiance in algae. J. Phycol. **27:** 8-14.

Fortunato, A. E., R. Annunziata, M. Jaubert, J.-P. Bouly, and A. Falciatore. 2015. Dealing with light: The widespread and multitasking cryptochrome/photolyase family in photosynthetic organisms. J. Plant Physiol. **172:** 42-54.

Gaston, K. J., J. Bennie, T. W. Davies, and J. Hopkins. 2013. The ecological impacts of nighttime light pollution: A mechanistic appraisal. Biol. Rev. **88:** 912-927.

Gaston, K. J., M. E. Visser, and F. Hölker. 2015. The biological impacts of artificial light at night: the research challenge. Phil. Trans. R. Soc. B **370:** 20140133.

Hale, J. D., G. Davies, A. J. Fairbrass, T. J. Matthews, C. D. F. Rogers, and J. P. Sadler. 2013. Mapping lightscapes: Spatial patterning of artificial lighting in an urban landscape. Plos One **8:** e61460.

Harris, T. D., and J. L. Graham. 2015. Preliminary evaluation of an in vivo fluorometer to quantify algal periphyton biomass and community composition. Lake Reserv. Manage. **31:** 127-133.

Hegemann, P., M. Fuhrmann, and S. Kateriya. 2001. Algal sensory photoreceptors. J. Phycol. **37:** 668–676.

Hieber, M., C. T. Robinson, U. Uehlinger, and J. V. Ward. 2005. A comparison of benthic macroinvertebrate assemblages among different types of alpine streams. Freshwat. Biol. **50:** 2087-2100.

Hill, W. R. 1996. Effects of light, p. 121-149. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Hölker, F. and others 2010a. The dark side of light: A transdisciplinary research agenda for light pollution policy. Ecol. Soc. **15:** 13.

Hölker, F., C. Wolter, E. K. Perkin, and K. Tockner. 2010b. Light pollution as a biodiversity threat. Trends Ecol. Evol. **25:** 681-682.

Hölker, F., C. Wurzbacher, C. Weissenborn, M. T. Monaghan, S. I. J. Holzhauer, and K. Premke. 2015. Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. Phil. Trans. R. Soc. B. **370:** 20140130.

Honnen, A.-C., P. R. Johnston, and M. T. Monaghan. 2016. Sex-specific gene expression in the mosquito *Culex pipiens f. molestus* in response to artificial light at night. BMC Genomics **17:** 22.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biom. J. **50:** 346-363.

Hudon, C., and E. Bourget. 1983. The effect of light on the vertical structure of epibenthic diatom communities. Bot. Mar. **26:** 317-330.

Jeffrey, S. W., R. F. C. Mantoura, and S. W. Wright (Eds.). 1997. Phytoplankton pigments in oceanography: Guidelines to modern methods. UNESCO Publishing, France.

Kahlert, M., and B. G. Mckie. 2014. Comparing new and conventional methods to estimate benthic algal biomass and composition in freshwaters. Environ. Sci Process Impacts **16:** 2627-2634.

Khoeyi, Z. A., J. Seyfabadi, and Z. Ramezanpour. 2012. Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae, *Chlorella vulgaris*. Aquacult. Int. **20:** 41-49.

Kianianmomeni, A., and A. Hallmann. 2014. Algal photoreceptors: In vivo functions and potential applications. Planta **239:** 1-26.

Kirk, J. T. O. 1994. Light and photosynthesis in aquatic ecosystems. Cambridge University Press.

Laviale, M., J. Prygiel, Y. Lemoine, A. Courseaux, and A. Creach. 2009. Stream periphyton photoacclimation response in field conditions: Effect of community development and seasonal changes. J. Phycol. **45:** 1072-1082.

Law, R. J. 2011. A review of the function and uses of, and factors affecting, stream phytobenthos. Freshw. Rev. **4:** 135-166.

Litchman, E. 1998. Population and community responses of phytoplankton to fluctuating light. Oecologia **117:** 247-257.

Longcore, T., and C. Rich. 2004. Ecological light pollution. Front. Ecol. Environ. **2:** 191–198.

Lowe, R. L., and Y. Pan. 1996. Benthic algal communities as biological monitors, p. 705-739. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Meyer, L. A., and S. M. P. Sullivan. 2013. Bright lights, big city: Influences of ecological light pollution on reciprocal stream-riparian invertebrate fluxes. Ecol. Appl. **23:** 1322-1330. 1

Mullineaux, C. W. 2001. How do cyanobacteria sense and respond to light? Mol. Microbiol. **41:** 965-971.

Oemke, M. P., and T. M. Burton. 1986. Diatom colonization dynamics in a lotic system. Hydrobiologia **139:** 153-166.

Okada, R., S. Kondo, S. B. Satbhai, N. Yamaguchi, M. Tsukuda, and S. Aoki. 2009. Functional characterization of CCA1/LHY homolog genes, PpCCA1a and PpCCA1b, in the moss *Physcomitrella patens*. Plant J. **60:** 551-563.

Pawson, S. M., and M. K. F. Bader. 2014. LED lighting increases the ecological impact of light pollution irrespective of color temperature. Ecol. Appl. **24:** 1561-1568.

Perkin, E. K., F. Hölker, K. Tockner, and J. S. Richardson. 2014a. Artificial light as a disturbance to light-naive streams. Freshwat. Biol. **59:** 2235-2244.

Perkin, E. K., F. Hölker, and K. Tockner. 2014b. The effects of artificial lighting on adult aquatic and terrestrial insects. Freshwat. Biol. **59:** 368-377.

Pinheiro, J., D. Bates, S. Debroy, D. Sarkar, and R Core Team. 2015. nlme: Linear and nonlinear mixed effects models. R package version 3.1-128, http://CRAN.R-project.org/package=nlme.

Poulin, C., F. Bruyant, M.-H. Laprise, A. M. Cockshutt, J. M.-R. Vandenhecke, and Y. Huot. 2014. The impact of light pollution on diel changes in the photophysiology of *Microcystis aeruginosa*. J. Plankton Res. **36:** 286-291.

R Core Team. 2015. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria.

Raven, J. A., and C. S. Cockell. 2006. Influence on photosynthesis of starlight, moonlight, planetlight, and light pollution (reflections on photosynthetically active radiation in the universe). Astrobiology **6:** 668-675.

Richardson, K., J. Beardall, and J. A. Raven. 1983. Adaptation of unicellular algae to irradiance: An analysis of strategies. New Phytol. **93:** 157-191.

Riley, W. D., B. Bendall, M. J. Ives, N. J. Edmonds, and D. L. Maxwell. 2012. Street lighting disrupts the diel migratory pattern of wild Atlantic salmon, *Salmo salar* L., smolts leaving their natal stream. Aquaculture **330:** 74-81.

Shatwell, T., A. Nicklisch, and J. Köhler. 2012. Temperature and photoperiod effects on phytoplankton growing under simulated mixed layer light fluctuations. Limnol. Oceanogr. **57:** 541-553.

Stevenson, R. J. 1996. An introduction to algal ecology in freshwater benthic habitats. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Sysoeva, M. I., E. F. Markovskaya, and T. G. Shiabaeva. 2010. Plants under continuous light: A review. Plant Stress **4:** 5-17.

Velez-Ramirez, A. I., W. Van Ieperen, D. Vreugdenhil, and F. F. Millenaar. 2011. Plants under continuous light. Trends Plant Sci. **16:** 310-318.

Wetzel, R. G. 2001. Limnology: Lake and river ecosystems, Third edition ed. Academic Press.

Woitke, P., C.-D. Martin, S. Nicklisch, and J.-G. Kohl. 1994. HPLC determination of lipophilic photosynthetic pigments in algal cultures and lake water samples using a non-endcapped C18-RP-column. Fresenius J. Anal. Chem. **348:** 762-768.

Zevenboom, W., and L. R. Mur. 1984. Growth and photosynthetic response of the cyanobacterium *Microcystis aeruginosa* in relation to photoperiodicity and irradiance. Arch. Microbiol. **139:** 232-239.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer.

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

Maja Grubisic[1,2], Gabriel Singer[1], M. Cristina Bruno[3], Roy H.A. van Grunsven[1], Alessandro Manfrin[1,2], Michael T. Monaghan[1], Franz Hölker[1]

[1] *Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 301/310, 12587 Berlin, Germany*

[2] *Institute of Biology, Freie Universität Berlin, Schwendenerstraße 1, 14195 Berlin, Germany*

[3] *Sustainable Ecosystems and Bioresources Department, Fondazione Edmund Mach, IASMA Research and Innovation Centre, Via Edmund Mach 1, 38010 San Michele all'Adige, Italy*

3.1. Abstract

Freshwaters are increasingly exposed to artificial light at night, yet the consequences for aquatic primary producers remain largely unknown. We used stream-side flumes to expose periphyton to artificial light at night. Pigment composition was used to infer community changes in lit and control periphyton before and after three weeks of treatment. Artificial nocturnal illumination decreased the proportion of red lineage taxa (diatoms and/or chrysophytes) by 14% and increased the proportion of cyanobacteria by 17% in spring periphyton, compared to the control. In autumn, effects were found for diatoxanthin, a non-specific pigment present in many algal groups and therefore could not be interpreted in a relation to any specific group. Diatoms in periphyton are highly nutritional and preferred food source for herbivores, in contrast to cyanobacteria. ALAN may thus decrease nutritional quality of periphyton, potentially decreasing food supply for higher trophic levels and secondary production in stream ecosystems.

3.2. Introduction

Due to urbanization and the spread of electrical lighting, freshwaters are increasingly exposed to artificial light at night (ALAN) worldwide (Falchi et al. 2016; Hölker et al. 2010). Several studies have reported the ecological effects of ALAN, but its impacts on freshwaters, particularly aquatic primary producers, remain understudied (Hölker et al. 2015; Perkin et al. 2011). In clear, shallow waters and small to mid-sized streams, benthic autotrophs within periphyton communities are important primary producers and form the base of the aquatic food

web (Stevenson 1996). Light is a key resource for autotrophs and regulates numerous physiological processes through circadian clocks (Hegemann et al. 2001). A recent study in a stream-side flume system, using an *in-situ* fluorometer (BenthoTorch) (Chapter 2) found that three weeks of exposure to ALAN decreased the biomass and increased the proportion of diatoms in periphyton in the early growth stages (up to three weeks). No effects of ALAN were detected for periphyton in later growth stages (three to six weeks). Here, we apply the more conventional method, high-performance liquid chromatography (HPLC) on the same periphyton communities in later growth stages and used pigment composition to assess periphyton community composition. HPLC pigment analysis is based on the separation of photosynthetic pigments and their degradation products and is suitable for the analysis of mixed algal assemblages (Millie et al. 1993). We expected that this method might reveal ALAN-induced changes in community composition in the later periphyton stages that were not detected with the *in-situ* fluorometry.

3.3. Methods

3.3.1. Experimental design and sampling procedure

The stream-side flume system used in this study, details of the experimental design and abiotic conditions are described in Chapter 2. 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. Artificial lights (LED strips, 3000 K, 20.3 ± 1.8 lux, mean and SD) were installed above either the upper or lower section (randomly chosen) 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 Before-After-Control-Impact (BACI) approach: four tiles were sampled from each of the ten flume sections before the start of experimental treatment (on March 31 and September 24) and after three weeks of experimental treatment (on April 23 and October 16). Environmental parameters (dissolved oxygen, temperature, pH, conductivity, velocity, turbidity) were measured at each sampling time and varied minimally between the flumes as described in Chapter 2 (Appendix A, Table S1).

3.3.2. Laboratory procedures

Periphyton was removed from the tiles and two aliquots were concentrated on GF/F glass-fibre filters (0.7 µm nominal pore size; Whatman Ltd., Maidstone, UK) for determination of dry mass and pigment composition. Filters for dry mass were dried to constant weight at 60°C and weighed. Filters for pigment analysis were transferred to -80°C for a minimum of 48 hours to stimulate cell lysis and subsequently freeze-dried and stored at -20°C. Pigments were analysed following the procedure described in Woitke et al. (1994) and Shatwell et al. (2012). Pigments were identified and quantified by their retention time and absorption spectra from standards and 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.

3.3.3. Data analysis

Pigment concentrations were normalized to the chl *a* concentration, z-standardized and subjected to principal component analysis (PCA) using functions from the packages vegan (Oksanen et al. 2015) and shape (Soetaert 2014) in R (Version 3.1.3., R Core Team 2015). PCAs were computed separately for each season. The values were log-transformed for autumn data to improve distributional properties of the data. 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 in LMM. A significant interaction treatment x time indicates an effect of ALAN on periphyton pigment composition. Pairwise comparisons of significant interactions were performed using the glht function from the multcomp package for R (Hothorn et al. 2008) with Benjamini-Hochberg *p* value adjustments.

3.4. Results

Eight pigments were identified in our samples (Table 3). Diatoxanthin was present only in spring periphyton and chl *b* and violaxanthin only in autumn. Chl *a* was the most abundant pigment in both lit and control periphyton and increased as periphyton developed with time in both seasons (time effect in spring: $F_{(1,70)} = 179.9$, $p < 0.001$; in autumn $F_{(1,70)} = 318.4$, $p < 0.001$). Chl *a* concentration 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 $F_{(1,70)} = 0.03$, $p = 0.86$).

Table 3. Pigment concentrations (µg cm^{-2}) measured by high-performance liquid chromatography (HPLC) in control periphyton (D) and lit periphyton (L) in the two seasons (mean ± SD, $n = 160$).

	Before		After	
Pigment	D	L	D	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

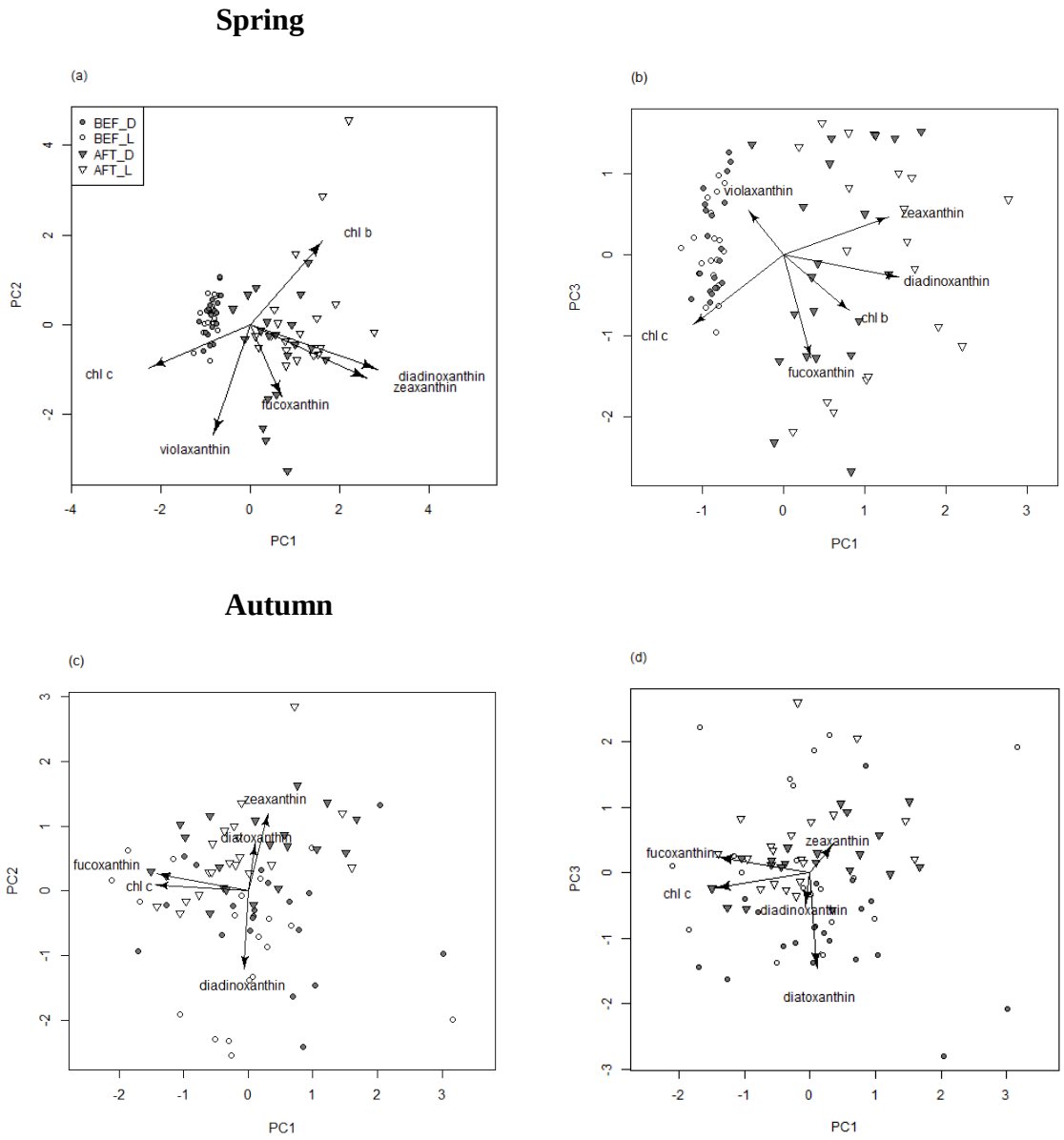

Figure 7. Correlation biplots produced by principal component analysis (PCA) based on relative pigment concentrations of periphyton in the two seasons (n = 80); before (BEF) and after (AFT) experimental treatment, in the control (D) and lit (L) periphyton. Plane of axes one (PC1) and two (PC2) (a, b) and two and three (PC3) (c, d) are shown. Amount of variation explained by the planes: (a) 64%, (b) 61%, (c) 59%, (d) 51%. Arrows show correlation between original pigment variables with PC axes; all correlations were significant ($p < 0.0001$).

In spring, the first three principal components explained 86% of the total variance, with axes explaining 39%, 25% and 22% of variation, respectively. Time and treatment-induced variation in pigment composition were visible along PC1 and PC2 (Fig.7a). PC1 correlated with diadinoxanthin (Pearson's r = 0.90), zeaxanthin (r = 0.82) and Chl *c* (r = -0.70);

its scores were significantly affected by ALAN (Fig. 8a; treatment x time $F_{(1,70)} = 8.76$, $p = 0.004$). Pairwise comparisons identified higher PC1 scores in lit periphyton after ALAN treatment ($p = 0.005$) compared to control periphyton, while no differences in scores were found before the treatment ($p = 0.42$). This indicated that relative concentrations of diadinoxanthin and zeaxanthin increased in lit periphyton relative to the control (12% and 17%, respectively), while Chl *c* decreased by 14% (Table 3). PC2 correlated with violaxanthin (Pearson's $r = -0.76$) and Chl *b* ($r = 0.58$), and PC 3 correlated 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 $F_{(1,70)} = 0.24$, $p = 0.62$).

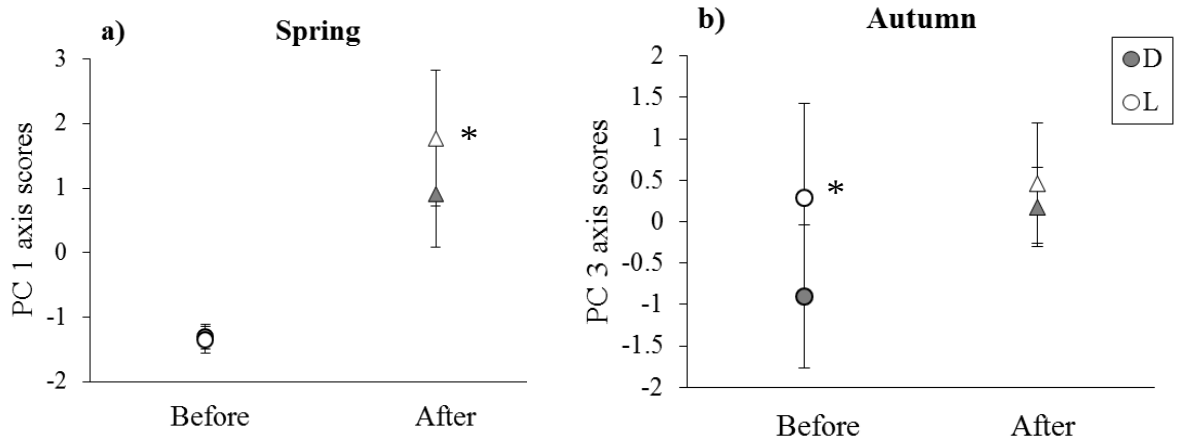

Figure 8. Scores of principal component axes that are significantly affected by the artificial light at night in the two seasons (mean ± SD); before and after the experimental treatment, in control (D) and lit (L) periphyton. Amount of variation explained by the PC axes: (a) 39%, (b) 20%. Asterisk indicates significant differences (LME, pairwise comparisons with Benjamini-Hochberg correction, $p = 0.004$).

In autumn, the first three principal components explained 78% of the total variance, with axes explaining 33%, 26% and 20% of variation, respectively. Time-induced variation in pigment composition was mainly visible along PC1 (Fig.7c), while a weak treatment-induced variation was limited to PC3 (Fig.7d). PC1 correlated with chl *c* (Pearson's $r = -0.89$) and fucoxanthin ($r = -0.91$), while 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. 8b; 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 ALAN treatment ($p = 0.24$). This indicates that previously 57% lower relative concentrations of diatoxanthin in lit periphyton (Table 1) evened out with concentrations found in control periphyton after the ALAN treatment.

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 and only in spring.

3.5. Discussion

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 sections indicated that biomass of autotrophs increased throughout the experiment, but no effects of ALAN on biomass were found. *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 (Chapter 2, Fig. 6c,d). Here, however, using a sensitive method, the HPLC pigment analysis, we were able to identify ALAN-induced changes in periphyton composition. ALAN affected different pigments in the two seasons. 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 represented by 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 compared to the control (12% and 17%, respectively). Because chl *b*, characteristic for green algae, was rarely detected, we conclude that changes in zeaxanthin suggest an increase in cyanobacteria. A taxonomic identification of periphyton community is necessary to further interpret observed changes in pigment composition. For the autumn communities, the observed changes in diatoxanthin, another non-specific pigment (Jeffrey et al. 1997) can only be interpreted as an overall community change. ALAN effects accounted for larger variation in spring periphyton (39% of explained variation) than on autumn (19%) and had different impacts in spring (14% decrease in chl *c*, 12% increase in diadinoxanthin and 17% increase in zeaxanthin compared to the control periphyton) than in autumn (57% higher diatoxanthin be-

fore the treatment). These seasonal differences are possibly due to the physiological acclimation driven by the light exposure history: spring communities tend to be adapted to low-light conditions of the preceding winter (Laviale et al. 2009), therefore periphyton in spring may be more sensitive to low-light levels such as those supplied by ALAN, than in autumn.

Pigment composition of autotrophs is strongly influenced by ambient light conditions. By increasing their cell pigment content algae are known to maximize photosynthetic efficiency under low light levels, a response known as "shade adaptation" (Falkowski and Raven 2013; Hill 1996). ALAN did not affect ratios of photosynthetic pigments to dry mass, which indicated that exposure to low-light levels at night did not result in physiological acclimation of the periphyton. Furthermore, the constant ratio of chl *a* to dry mass indicated that the ratio of autotrophs to heterotrophs in the periphyton was also not affected by ALAN.

Periphytic algae are the major basal food source in streams and by decreasing the abundance of red lineage algae and increasing the abundance of cyanobacteria ALAN may decrease nutritional quality of periphyton for herbivores. Diatoms are preferred food source for invertebrate grazers because of their high content of omega-3 polyunsaturated fatty acids (PUFA) and complex polysaccharides (see Guo et al. 2016; Guschina and Harwood 2009). Chrysophytes are also an important food source for stream consumers (Nicholls and Wujek 2003). In contrast, cyanobacteria are considered as low-quality food because as they lack important PUFAs and sterols. It was previously found that ALAN non-linearly affected the proportion of diatoms in stream periphyton in early developmental stages, where it decreased (by 11% after two weeks) and increased (by 5% after three weeks) their proportion in the community in autumn. ALAN was also found to decrease the proportion of cyanobacteria by 54% in periphyton in early developmental stages in spring. The changes in pigments found here for periphyton in later developmental stages (three to six weeks) indicate that ALAN decreased the proportion of diatoms and/or chrysophytes by 14% and increased the proportion of cyanobacteria by 17% in stream periphyton in spring. Algal food quality has been recognized as an important regulator of trophic efficiency of energy flow through stream ecosystems (see Cashman et al. 2013; Guo et al. 2016) and by altering the nutritional quality of primary consumers, ALAN may affect food supply for higher trophic levels and food web dynamics in stream ecosystems.

3.6. Conclusions

Periphyton plays a key role in primary production, nutrient cycling and food web processes in many small and mid-sized streams and clear, shallow waters (Law 2011; Stevenson 1996), thus the community-level changes induced by ALAN may alter species dominance patterns, species dynamics and diversity and potentially have consequences for higher trophic levels through changes in food quality and quantity (Feminella and Hawkins 1995; Perkin et al. 2011). It was previously reported that ALAN can alter the proportion of diatoms and cyanobacteria in stream periphyton in early developmental stages (up three weeks). Here we found that ALAN significantly altered proportions of diatoms and/or chrysophytes and cyanobacteria also in later developmental stages (three to six weeks), the effect that was missed with the rapid *in situ* analysis. 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, two groups of primary producers with contrasting nutritional quality in the periphyton, ALAN may affect the availability of essential omega-3 polyunsaturated fatty acids for stream consumers. The consequences for food web dynamics and secondary production in streams remain unknown.

Acknowledgments

We wish to thank Barbara Stein (IGB) for performing HPLC analysis and Cécile Périllon (IGB) for advice on spectrogram analysis. This work has been carried out within the Erasmus Mundus Joint Doctorate Program SMART, funded by the EACEA, the Federal Ministry of Research and Technology, Germany (BMBF-033L038A) and the Federal Agency for Nature Conservation, Germany (3514821700).

References (Chapter 3)

Cashman, M. J., J. D. Wehr, and K. Truhn. 2013. Elevated light and nutrients alter the nutritional quality of stream periphyton. Freshwat. Biol. **58:** 1447–1457.

Falchi, F. and others 2016. The new World Atlas of artificial night sky brightness. Sci. Adv. **2:** e1600377.

Falkowski, P. G., and J. A. Raven. 2013. Aquatic photosynthesis. Princeton University Press.

Feminella, J. W., and C. P. Hawkins. 1995. Interactions between stream herbivores and periphyton: A quantitative analysis of past experiments. J. N. Am. Benthol. Soc. **14:** 465-509.

Guo, F., M. J. Kainz, F. Sheldon, and S. E. Bunn. 2016. The importance of high-quality algal food sources in stream food webs – current status and future perspectives. Freshwat. Biol. **61:** 815–831.

Guschina, I., and J. L. Harwood. 2009. Algal lipids and effect of the environment on their biochemistry, p. 1-24. *In* M. T. Arts, M. T. Brett and M. J. Kainz [eds.], Lipids in aquatic ecosystems. Springer.

Hegemann, P., M. Fuhrmann, and S. Kateriya. 2001. Algal sensory photoreceptors. J. Phycol. **37:** 668–676.

Hill, W. R. 1996. Effects of light, p. 121-149. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Hölker, F. and others 2010. The dark side of light: A transdisciplinary research agenda for light pollution policy. Ecol. Soc. **15:** 13.

Hölker, F., C. Wurzbacher, C. Weissenborn, M. T. Monaghan, S. I. J. Holzhauer, and K. Premke. 2015. Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. Phil. Trans. Roy. Sci. B. **370:** 20140130.

Hothorn, T., F. Bretz , and P. Westfall. 2008. Simultaneous inference in general parametric models. Biom. J. **50:** 346-363.

Jeffrey, S. W., R. F. C. Mantoura, and S. W. Wright (eds.). 1997. Phytoplankton pigments in oceanography: Guidelines to modern methods. UNESCO Publishing, France.

Laviale, M., J. Prygiel, Y. Lemoine, A. Courseaux, and A. Creach. 2009. Stream periphyton photoacclimation response in field conditions: Effect of community development and seasonal changes. J. Phycol. **45:** 1072-1082.

Law, R. J. 2011. A review of the function and uses of, and factors affecting, stream phytobenthos. Freshw. Rev. **4:** 135-166.

Millie, D. F., H. W. Paerl, and J. P. Hurley. 1993. Microalgal pigment assessments using high-performance liquid chromatography: A synopsis of organismal and ecological applications. Can. J. Fish. Aquat. Sci. **50:** 2513-2527.

Nicholls, K. H., and D. E. Wujek. 2003. Chrysophycean Algae. *In* J. D. Wehr and R. G. Sheath [eds.], Freshwater algae of North America: Ecology and classification. Academic Press.

Oksanen, J. and others 2015. vegan: Community ecology package. R package version 2.4-2. https://CRAN.R-project.org/package=vegan.

Perkin, E. K., F. Hölker, J. S. Richardson, J. P. Sadler, C. Wolter, and K. Tockner. 2011. The influence of artificial light on stream and riparian ecosystems: Questions, challenges, and perspectives. Ecosphere **2:** 1-16.

Shatwell, T., A. Nicklisch, and J. Köhler. 2012. Temperature and photoperiod effects on phytoplankton growing under simulated mixed layer light fluctuations. Limnol. Oceanogr. **57:** 541-553.

Soetaert, K. 2014. shape: Functions for plotting graphical shapes, colors.

Stevenson, R. J. 1996. An introduction to algal ecology in freshwater benthic habitats. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

R Core Team. 2015. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria.

Woitke, P., C.-D. Martin, S. Nicklisch, and J.-G. Kohl. 1994. HPLC determination of lipophilic photosynthetic pigments in algal cultures and lake water samples using a non-endcapped C18-RP-column. Fresenius J. Anal. Chem. **348:** 762-768.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer.

4. DNA metabarcoding the 18S rRNA V4 region to investigate effects of artificial light at night on stream periphyton

Maja Grubisic[1,2], Jonas Zimmermann[3], Christian Wurzbacher[1,4,5], Sebastian Proft[3,5], Franz Hölker[1*], Michael T. Monaghan[1,4,5*]

[1] *Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 301/310, 12587 Berlin, Germany*

[2] *Institute of Biology, Freie Universität Berlin, Schwendenerstraße 1, 14195 Berlin, Germany*

[3] *Botanical Garden and Botanical Museum, Freie Universität Berlin, Königin-Luise-Straße 6-8, 14195 Berlin, Germany*

[4] *University of Gothenburg, Medicinaregatan 18, 41390 Gothenburg, Sweden*

[5] *Berlin Center for Genomics in Biodiversity Research, Königin-Luise-Straße 6-8, 14195 Berlin, Germany*

**authors contributed equally*

4.1. Abstract

Periphyton communities, especially periphytic diatoms, are valuable indicators of environmental conditions in lakes and running waters because they are sensitive to physical, chemical and biological changes (Lowe and Pan 1996). Diatoms can grow under a wide range of light intensities, including low-light environments such as heavy shade or under ice (Allan and Castillo 2007; Smol and Stoermer 2010), although individual taxa vary in their photosynthetic optima (Hill 1996) and thus potentially their capability to use low-level artificial light at night for photosynthesis and growth. We performed a field experiment in stream-side flume system on a sub-alpine stream to test whether natural periphyton communities would respond to artificial light at night (20 lux, white LED) by increasing photosynthetic production or by changes in taxonomic composition and relative abundance. We used DNA metabarcoding of V4 region of the 18S rRNA gene to determine community composition and compared communities that were exposed to artificial nocturnal illumination with those exposed to natural light-dark cycles. The study followed a replicated Before-After-Control-Impact (BACI) design and periphyton was exposed to artificial light at night over three weeks, after having four weeks of development under a natural light regime. We conducted the experiment once in spring and once in autumn. Artificial nocturnal illumination had no significant effect on periphyton diversity and qualitative composition. Differences in species abundance (based on

read numbers) were measurable at the community level only in autumn. Three taxa responded to the treatment: one chrysophyte (a *Spumella*-like flagellate) and one diatom (*Diatoma hyemalis*) increased in abundance in the lit treatment, while one chrysophyte taxa (*Hydrurus foetidus*) decreased in the abundance in the lit treatment, compared to the control periphyton. Our results indicate that periphyton communities respond to artificial nocturnal illumination; it was dependent on season, taxon-specific and not limited to autotrophs. These results emphasize the need for a better mechanistic understanding of the impacts of artificial light at night on periphyton communities and potential implications for aquatic food webs and ecosystem processes.

4.2. Introduction

Periphytic diatoms are commonly used for biological monitoring of environmental conditions in rivers, streams and shallow lakes (Bennion et al. 2010; Lowe and Pan 1996). Diatoms are often the most diverse and abundant group in periphyton; they have short generation times and quickly respond to environmental alterations by changing their growth rates, leading to differences in biomass and species composition of periphyton (Stevenson et al. 2010). Diatoms in periphyton are not only species-rich but also physiologically diverse, where each species is characterized by its own set of environmental preferences and tolerances (Lowe and Pan 1996). Species with narrow optima and tolerances for environmental variables respond sensitively to changes in physical, chemical or biological disturbances such as temperature, nutrient levels and grazing (Stevenson et al. 2010 and references therein). Environmental conditions interact to differentially affect physiological performance of diatom species, that manifests in the presence/absence and abundance of individual taxa and the composition of diatom communities (Stevenson et al. 2010).

Alteration of natural light regime by artificial light at night (ALAN) might affect periphyton taxonomic diversity and composition, because intensity and quality of light are proximate factors that directly influence primary producers (Biggs 1996; Wu 2017). Light preferences generally differ between major taxonomic groups, e.g. diatoms and cyanobacteria are better adapted to grow under low light levels than green algae (Langdon 1988; Richardson et al. 1983). Within major taxonomic groups there is a considerable interspecific variability in minimum light requirements and optima, but autecological light requirements of individual benthic species are largely unknown because growth versus irradiance measurements have rarely been made (Hill 1996). Diatoms are able to grow under a wide range of light intensities

including low light conditions (Richardson et al. 1983) and this feature may provide them with a competitive advantage over other algal groups in light-polluted environments that receive high light levels during the day and low light levels during the night (Hölker et al. 2015). One year of artificial nocturnal illumination (6.8 – 8.5 lux by high-pressure sodium light) increased abundance of some diatoms and cyanobacteria in microbial communities in illuminated sediments (Hölker et al. 2015) and a laboratory incubations of the same microbial communities (under 71 lux by LED) indicated that nocturnal photosynthesis was taking place under ALAN. Diatoms seem to be able to utilize light supplied by artificial nocturnal illumination as a source of energy for nocturnal photosynthesis. We thus hypothesized that ALAN (20 lux, LED in our experiment) would stimulate growth of diatoms and increase their proportion in ALAN-exposed periphyton compared to periphyton grown under natural light regime. Taxon-specific differences in efficiency of utilization of low-light levels might favor certain diatom taxa over others, resulting in alteration of their abundance and taxonomic composition of periphyton. Furthermore, seasonal patterns in environmental conditions, especially temperature and irradiance, strongly drive seasonal variation in periphyton community composition in temperate latitudes (Biggs 1996). Thus the sensitivity and response of periphyton to ALAN was expected to differ between the seasons.

The recent development of next-generation sequencing technologies allows for DNA metabarcoding, a high-throughput amplicon sequencing of community DNA. Sequences are clustered to form operational taxonomic units (OTUs) and these are identified from environmental samples based on the assignment of short DNA sequences (DNA barcodes) of unidentified taxa to a reference database (Kermarrec et al. 2014). Metabarcoding is widely recognized as a fast and reliable approach for species identification (Visco et al. 2015), evaluation of community composition (Zimmermann et al. 2011) and diversity in a target environment suitable for environmental monitoring (Pawlowski et al. 2016). Metabarcoding has been applied for assessment of the diversity and composition of microbial (e.g. Degnan and Ochman 2012; Hölker et al. 2015) and diatom communities (e.g. Visco et al. 2015).

Here, we used Illumina MiSeq high-throughput sequencing to analyze effects of ALAN (20 lux, LED) on taxonomic composition and diversity of stream periphyton communities (three to six week old) using the V4 region of the 18S rRNA gene as a DNA barcode and primers targeting diatoms but known to amplify other groups of algae. We compared taxonomic composition and abundance of periphyton exposed to three weeks of ALAN treatment to those of control periphyton that experienced a natural light regime. The experiment was

conducted in a stream-side flume system along a sub-alpine stream in two seasons, spring and autumn.

4.3. Methods

4.3.1. Experimental design and sampling

The stream-side flume system used in this study, the details of the experimental design and abiotic conditions are described in detail in Chapter 2. The flumes are positioned at the right bank of Fersina stream (Trentino, Italy, 46° 04′ 32″ N, 11° 16′ 24″ E) and constantly fed with water from the stream throughout the experimental period. Five U-shaped flumes (labelled A to E) are 20 m long and 30 cm wide. For this analysis, only samples from flumes A to C (wall height 30 cm) were analyzed. 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 under a natural light regime. Artificial lights (LED strips, 3000 K, light intensity measured at the water surface 20.3 ± 1.8 lux, mean and SD) were installed above either the upper or the lower section (randomly selected) 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. One tile with periphyton from each flume section was randomly chosen for taxonomic analysis with DNA metabarcoding. The experiment followed a replicated Before-After-Control-Impact (BACI) design: the tiles were sampled from each of the six flume sections before the start of experimental treatment (on March 31 and September 24) and after three weeks of experimental treatment (on April 23 and October 16). This allowed us to compare periphyton community developed in lit (L) sections under artificial light at night, with that from control (D) sections exposed to natural cycles of light and dark, with three biological replicates. Environmental parameters (dissolved oxygen, temperature, pH, conductivity, velocity, turbidity) were measured at each sampling time and varied minimally between the flumes, as described in Chapter 2 (Appendix A, Table S1).

The details of sampling are given in Chapter 2. Briefly, periphyton was brushed from the tiles using a toothbrush and washed into a suspension using pre-filtered flume water (GF/F glass-fibre filters, 0.7 µm nominal pore size, Whatman Ltd., Maidstone, UK). Samples were transported in the dark and processed in the laboratory within 12 hours. In the laboratory, aliquots of periphyton suspension were concentrated on Whatman GF/F glass-fibre filters using

vacuum filtration. Filters were placed in a 1.5 mL screw-cap microcentrifuge tube, filled with pure ethanol and stored at 4°C until further analysis.

4.3.2. DNA metabarcoding analysis

Filters were taken from the ethanol and air-dried on a tissue paper before further processing. DNA was extracted using a NucleoSpin Plant II kit (Macherey-Nagel Gmbh & Co., Düren, Germany). The filters were initially incubated in PL1 lysis buffer at 65°C for two hours and further steps were performed according to the manufacturer's instructions. DNA concentrations were quantified using a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, California, USA) and normalized to a concentration of 20 ng μL^{-1}. The V4 region of the 18S rRNA gene was amplified using the primers DIV4for: 5′-GCGGTAATTCCAGCTCCAATAG-3′ and DIV4rev3: 5′-CTCTGACAATGGAATACGAATA-3′ (Biomers, Ulm, Germany). These primers were developed by Zimmermann et al. (2011) for identification of diatom taxa and modified by Visco et al. (2015) for use on the Illumina sequencing platform. PCR amplifications were performed in a total volume of 25 µL using 0.4 µL Herculase II Fusion DNA Polymerase (Agilent Technologies Inc., Santa Clara, California, USA), 1.5 µL BSA (10 pm μL^{-1}), 0.25 µL DMSO, 1 µL of each forward and reverse primer (10 pm μL^{-1}), 0.5 µL dNTP mix (25 mM each dNTP), 5 µL Herculase II reaction buffer and 1 µL of template DNA (20 ng μL^{-1}). PCA regime was modified from Visco et al. (2015) to include an initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 45 seconds, annealing at 52°C for 45 seconds and elongation at 72°C of 1 minute; and a final elongation at 72°C for 10 minutes. PCR products were visualized on 1% agarose gel using gel electrophoresis and a random subsample of 6 PCR products was quantified using then Agilent High Sensitivity DNA Analysis kit (Agilent Technologies Inc.). Products of two PCR reactions were pooled into a final volume of 50 µL. An aliquot of 25 µL of PCR amplicons was purified using HighPrep PCR paramagnetic beads (Magbio Genomics, Gaithersburg, Maryland, USA). The indexing PCR regime, in which individual tags were attached to the 5' end of the primer, included denaturation at 95°C for 120 seconds, 8 cycles of denaturation at 95°C for 20 seconds, annealing at 52°C for 30 seconds and elongation at 72°C for 30 seconds, followed by elongation at 72°C for 180 seconds. Products were purified using HighPrep PCR paramagnetic beads and quantified using Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, California, USA). Library preparation was performed using MiSeq Reagent Kit V3 (Illumina, San Diego, Cali-

fornia, USA) following manufacturer instructions. Sequencing was performed using an Illumina MiSeq sequencing platform.

4.3.3. Data analysis

OTU assignment was performed using the UPARSE pipeline (Edgar 2013) with 97% similarity cutoff and the output was arranged as a taxon-abundance table. Data were analyzed using the vegan package (Oksanen et al. 2015) for R (Version 3.1.3, R Core Team 2015). To make samples with different numbers of reads comparable, read abundance data was randomly subsampled to the smallest number of reads found across samples (function rrarefy). Both subsampled and full data matrices were used to calculate alpha diversity estimates (i.e. chao taxa richness, evenness of taxa abundances and Shannon index) and those were visually explored for linearity and tested for correlation to assess their dependency on sample read numbers. The subsampled (rarified) matrix was subjected to non-metric multidimensional scaling analysis (nMDS) based on dissimilarity matrix calculated from Bray-Curtis distances. Additionally, the subsampled matrix was transformed to presence-absence matrix and subjected to the nMDS analysis based on Euclidean distances. The resulting scores of nMDS axes were used as estimates of quantitative (from read abundance) and qualitative (from presence-absence data) differences in taxonomic composition between periphyton in the two experimental seasons and between lit and control periphyton within each season. The scores of nMDS axes and diversity indices were statistically tested using linear mixed model (LMM) (Zuur et al. 2009) available in the nlme package (Pinheiro et al. 2015) for R. Time ("before" and "after") and treatment ("lit" and "control") were included as fixed factors in the model and flume was included as a random factor to account for spatial dependency between the tiles sampled from two sections of the same flumes. When the observed variance differed between the levels of fixed factors, these were used as variance covariates (Zuur et al. 2009). Pairwise comparisons of significant interactions were performed using the glht function from the multcomp package for R (Hothorn et al. 2008) with Benjamini-Hochberg *p* value adjustments. Because the experimental design followed a replicated BACI approach, an effect of ALAN is represented by the interaction term treatment x time. In addition we performed a permutation analysis to identify responses of single taxa to ALAN. A function in R was written to randomly subsample the read abundance matrix 1000 times and perform a Kruskal-Wallis test per each taxonomic group for each subsampling. *Pq* values show the fraction of

non-significant Kruskal-Wallis tests in 1000 subsamplings. The significance level for the *Pq* value was set to 0.05 without correction for multiple comparisons.

OTU taxonomic assignment was performed using BLAST (Altschul et al. 1990).

Taxonomic composition of periphyton is visualized via Krona charts (Ondov et al. 2011) based on read abundance matrix with read numbers averaged for treatment x time groups (n = 3) for spring periphyton (see Appendix B, Fig. S2) and autumn periphyton (Fig. S3). Visualization is based on taxonomic assignment using OBI tools pipeline (Boyer et al. 2016) with maximum depth of 11.

4.4. Results

OTU clustering resulted in samples with different numbers of reads (24760 ± 12616, mean and SD). The smallest sample had 1154 reads and all samples were randomly subsampled to this sample size for better comparability. From the total of 632 OTUs (hereafter referred to as taxa) identified in the original matrix, 470 taxa were found in spring and 503 taxa in autumn periphyton. Subsampling reduced these numbers to a total of 237 taxa, of which 138 were present in spring and 179 in autumn periphyton.

Table 4. Alpha diversity estimates for lit (L) and control (D) periphyton before and after three weeks of exposure to artificial light at night in the two seasons. Calculations based on the read abundance data matrix subsampled to the smallest number of reads (1154) (n = 48).

		Richness	Evenness	Shannon index
	Spring			
Before	D	131.00	0.13	0.45
	L	148.03	0.09	0.27
After	D	146.39	0.07	0.28
	L	88.00	0.14	0.43
	Autumn			
Before	D	201.01	0.46	1.86
	L	148.23	0.46	1.83
After	D	115.49	0.32	1.13
	L	149.49	0.37	1.38

4.4.1. Periphyton diversity and quantitative composition

Chao richness estimates calculated from the subsampled data matrix with read abundances (Table 4) were not correlated with chao richness estimates from the full data matrix (Pearson's r = 0.65, p = 0.08). This indicated that richness estimates were dependent on sample read numbers, thus they were considered unreliable and were not analyzed further.

Both Shannon diversity index (r = 0.99, p <0.0001) and community evenness (r = 0.99, p <0.001) strongly correlated between the subsampled and full data matrices and those based on the subsampled matrix were statistically tested. Shannon diversity was lower in spring periphyton (0.36 ± 0.21, mean and SD averaged for season) than in autumn (1.55 ± 0.43; LMM: $F_{1,6}$ = 83.03, p < 0.0001) but there were no differences between lit and control periphyton after ALAN treatment in either season (LMM: treatment x time $F_{1,6}$ = 1.64, p = 0.25 in spring, $F_{1,6}$ = 0.62, p = 0.46 in autumn). Evenness was also lower in spring periphyton (0.10 ± 0.06, mean and SD) compared to autumn (0.40 ± 0.09, LMM: $F_{1,6}$ = 93.97, p < 0.0001) but it was not affected by ALAN in either season (LMM: treatment x time $F_{1,6}$ = 2.51, p = 0.16 in spring, $F_{1,6}$ = 0.34, p = 0.58 in autumn).

nMDS ordination based on the subsampled matrix with read abundances showed that periphyton differed in quantitative composition between spring and autumn (Fig. 9; LMM: nMDS1 $F_{1,6}$ = 27.00, p < 0.0001, nMDS2 $F_{1,6}$ = 7.92, p = 0.01). Spring periphyton consisted of 138 taxa, while 179 taxa were present in autumn communities.

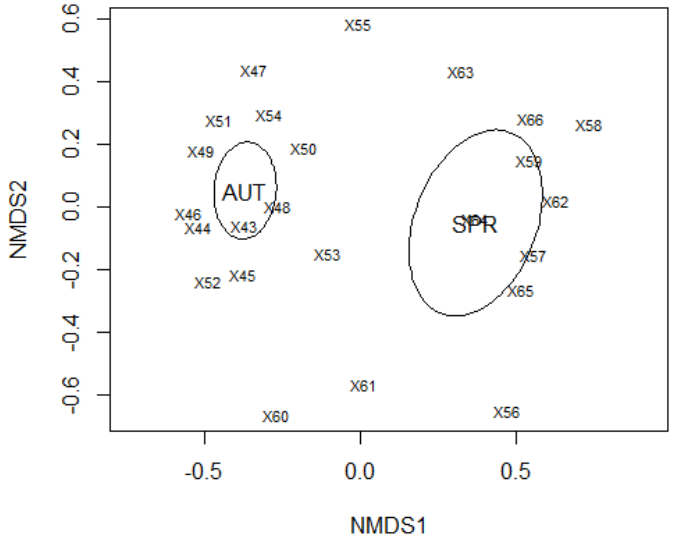

Figure 9. Ordination plot of non-metric multidimensional scaling (nMDS) based on the dissimilarity matrix calculated from subsampled read abundance data with Bray-Curtis distances

(*n* = 48). Ellipses show 95% confidence intervals around group centroids for spring (SPR) and autumn (AUT) periphyton communities. Sites represent individual samples.

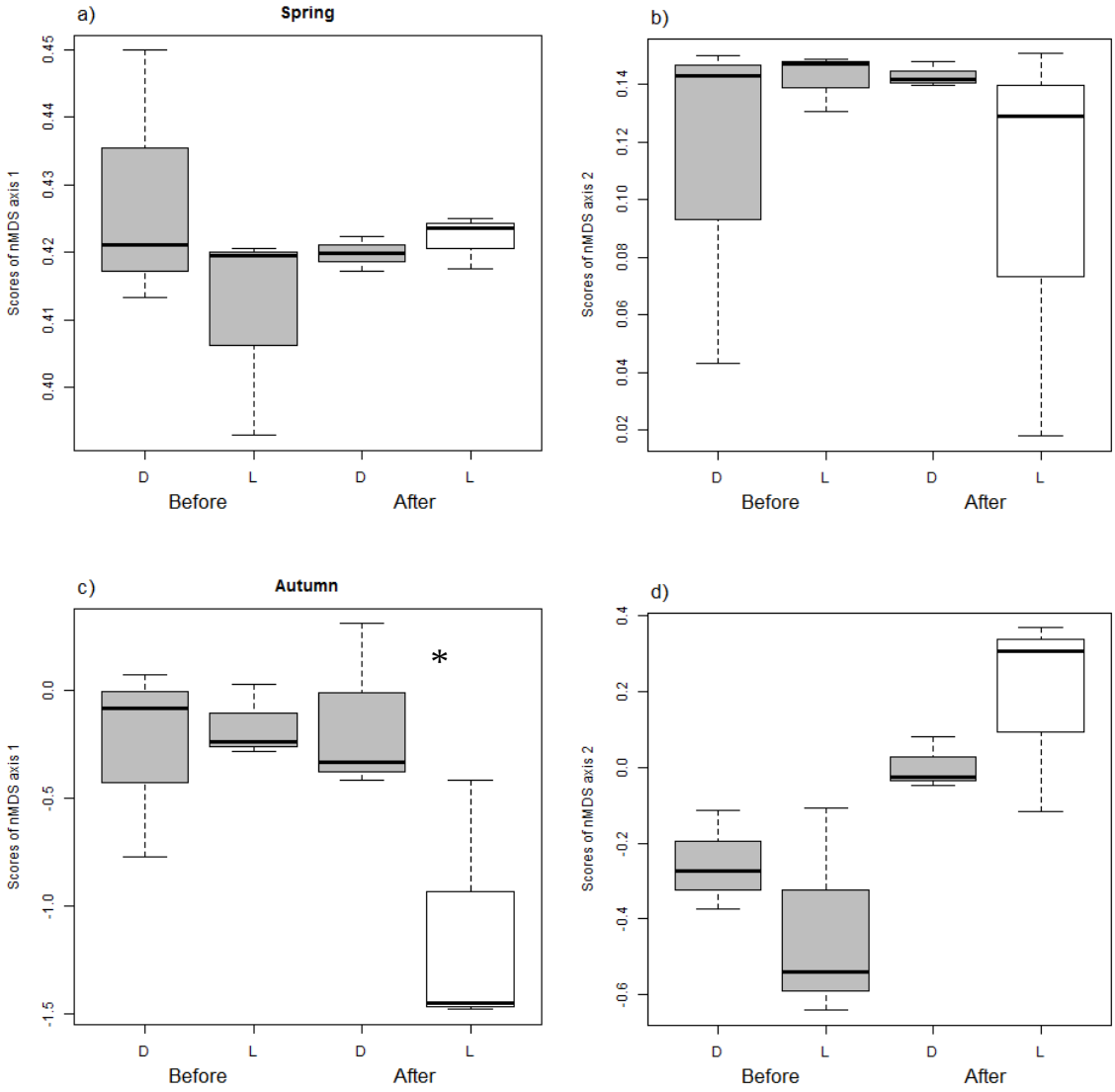

Figure 10. Scores of the non-metric multidimensional scaling (nMDS) ordination based on the dissimilarity matrix calculated from subsampled read abundance data with Bray-Curtis distances across lit (L) and control (D) periphyton, before and after three weeks of exposure to artificial nocturnal illumination (*n* = 48). An asterisk indicates significant effect of ALAN ($p < 0.05$).

The abundances of taxa in periphyton were not affected by ALAN in spring (Fig. 10a,b; LMM: factor treatment x time nMDS1 $F_{1,6} = 1.86$, $p = 0.22$, nMDS2 $F_{1,6} = 1.88$, $p = 0.22$), but the abundances of taxa in periphyton were significantly affected by ALAN in autumn (Fig. 10c,d; LMM: factor treatment x time nMDS1 $F_{1,6} = 6.01$, $p = 0.0497$, nMDS2 $F_{1,6}$

= 2.66, p = 0.15). Pairwise comparisons for nMDS1 scores showed that periphyton from lit and control sections did not differ before the ALAN treatment (Fig. 11b; p = 0.90), but after the treatment there were significant differences in taxa abundance between lit and control periphyton (p = 0.006).

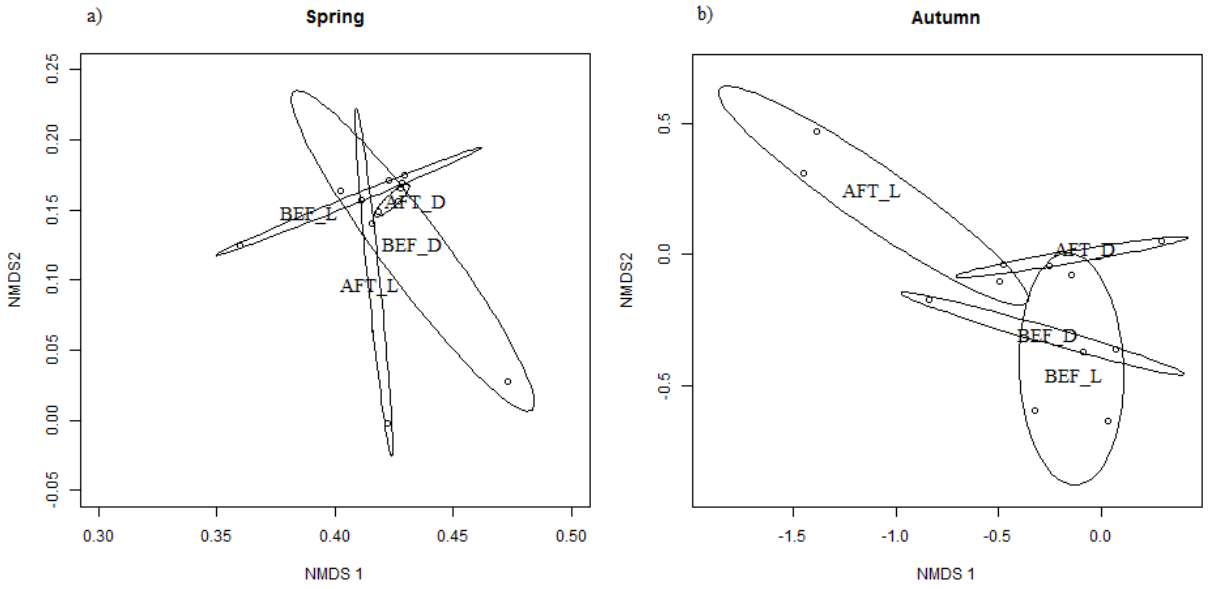

Figure 11. Ordination plots of non-metric multidimensional scaling (nMDS) based on the dissimilarity matrix calculated from subsampled read abundance data with Bray-Curtis distances. The data is shown separately for the two seasons. Ellipses show 95% confidence intervals around group centroids for treatment (lit, L and control, D) x time (before, BEF and after, AFT) groups (n = 3).

4.4.2. Qualitative composition

Similarly to the quantitative data, nMDS ordination based on the subsampled matrix with presence-absence data showed that periphyton had different qualitative taxonomic composition in spring and autumn (Fig. 12; LMM: nMDS1 $F_{1,6}$ = 18.33, p < 0.0001, nMDS2 $F_{1,6}$ = 3.36, p = 0.08).

The taxonomic composition of periphyton represented by nMDS scores was not affected by ALAN neither in spring (Fig. 13a,b; LMM: factor treatment x time nMDS1 $F_{1,6}$ = 0.46, p = 0.52, nMDS2 $F_{1,6}$ = 2.16, p = 0.19) nor in autumn (Fig. 13c,d; GLS: factor treatment x time nMDS1 $F_{1,1,}$ = 0.34, p = 0.58, nMDS2 $F_{1,6}$ = 0.07, p = 0.81).

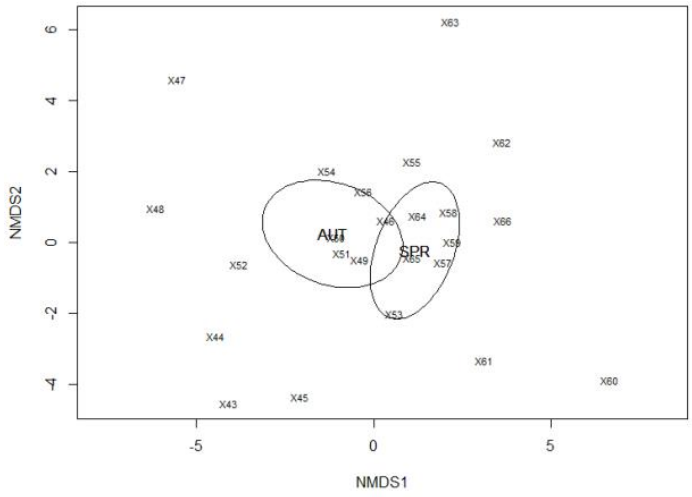

Figure 12. Ordination plot of non-metric multidimensional scaling (nDMS) based on the dissimilarity matrix calculated from subsampled presence-absence data with Euclidean distances (n = 48). Ellipses show 95% confidence intervals around group centroids for spring (SPR) and autumn (AUT) periphyton communities. Sites represent individual samples.

4.4.3. Effects on single taxa

Permutation analysis indicated that periphyton communities from spring and autumn differed in the relative abundance of 13 OTUs that were identified in the database as 9 taxa (Appendix B, Table S3). No differences were found between periphyton from lit and control sections before the treatment (Pq > 0.5). In autumn, three taxa responded to the ALAN treatment. The chrysophyte *Hydrurus foetidus* (98% similarity with sequence in NCBI database), the dominant taxon represented by 48 - 52% of the reads in periphyton before the treatment (i.e. 8328 - 14546 reads in the full data matrix), decreased by 70% in relative abundance in lit periphyton compared to the control (Pq < 0.0001). A diatom *Diatoma hyemalis* (99% similarity with database sequence) and a chrysophyte taxon identified by BLAST search as a *Spumella*-like flagellate (99% similarity with database sequence) decreased in the periphyton with time, but their abundancies were higher in lit periphyton after ALAN treatment compared to the control (72% for *D. hyemalis*, Pq < 0.001 and 66% for the *Spumella*-like flagellate, Pq = 0.005). Both *D. hyemalis* and the *Spumella*-like flagellate were represented in average by less than 1% of the total read number in the full data matrix (i.e. 0.2 – 0.9% or 35 – 245 reads for *D. hyemalis* and 0.04 – 0.2% or 8 - 58 reads for *Spumella*-like flagellate). *H. foetidus* was a dominant taxon also in spring periphyton accounting for 90 - 97% or reads in the community (i.e. 19283 – 35180 reads in the full data matrix) and a weak trend in decreased abundance in lit periphyton was found (32% decrease); however the change was not

statistically significant (Pq = 0.068). Other taxa were not affected by ALAN in spring periphyton.

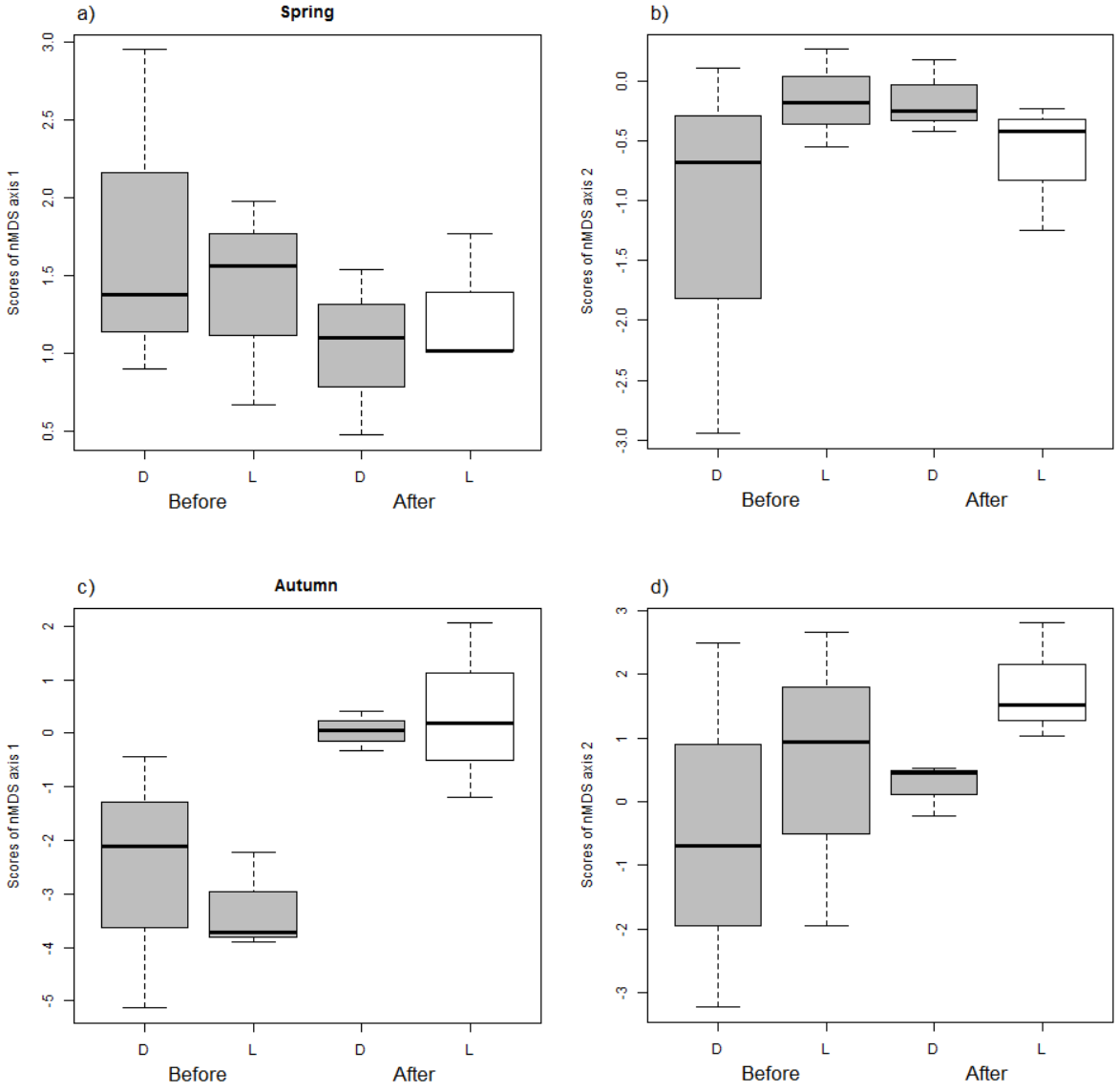

Figure 13. Scores of the non-metric multidimensional scaling (nMDS) ordination based on the dissimilarity matrix calculated from the subsampled presence-absence data with Euclidean distances across lit (L) and control (D) periphyton, before and after three weeks of exposure to artificial nocturnal illumination (n = 48).

4.5. Discussion

Artificial light at night can potentially stimulate growth of primary producers in periphyton that are able to efficiently utilize low-light levels, resulting in their increased abundance and an alteration of periphyton taxonomic composition. We found that relatively low-

level artificial nocturnal illumination (20 lux, LED) affected quantitative composition of periphyton in autumn, but did not affect diversity and presence/absence of species in the community. One diatom and two chrysophyte taxa, of which one autotroph and one heterotroph, were found to respond to ALAN in a species-specific manner. In spring, no effects of ALAN on periphyton were found, on the level of single taxa or the community. We did not observe an increase in abundance of diatom taxa as hypothesized, but we found a taxon-specific response, with some taxa increasing and some decreasing under artificial nocturnal illumination.

The primers that were applied in our study were developed for molecular identification of diatoms (Visco et al. 2015; Zimmermann et al. 2011) and our choice of primers was based on our expectation that diatoms would respond to ALAN because of their ability to successfully grow in low-light environments. The primers also amplified DNA from other groups such as chrysophytes (gold-brown algae) and chlorophytes (green algae), and those were kept for the analysis of complete diversity that was captured by the primers.

Chrysophytes are closely related to diatoms, both belonging to Stramenopiles, a monophyletic group of heterokont algae that also includes red algae (Andersen 2004). These "red lineage algae" are characterized by chlorophyll *c* and fucoxanthin that are often used as marker pigments. Chrysophytes are widely distributed in freshwater systems, typically associated with waters with low to moderate productivity, low alkalinity and conductivity (Nicholls and Wujek 2003). In comparison to other abiotic factors, effects of light on chrysophytes are less known and most taxa grow best at moderate light intensities (e.g. 200 to 800 µmol m^{-2} s^{-1}, for comparison with 0.31 µmol m^{-2} s^{-1} ALAN in our experiment). At low light intensities, many diatoms, cyanobacteria and red algae may grow better than chrysophytes (Hill 1996 and references therein), but light requirements of individual taxa are generally unknown (Nicholls and Wujek 2003). *H. foetidus* is a macroscopic alga that forms branched, gelatinous colonies on rocks and firm substrates in clear, flowing waters of alpine and subalpine streams worldwide (Nicholls and Wujek 2003; Parker et al. 1973). It is a cold-water stenotherm that grows abundantly during snowmelt and from late winter until early summer and starts to decline when the temperature rises above 10 C; its cells can develop a flagellum and swim away or be carried downstream. In streams with consistently low temperatures it can be found throughout summer and autumn (see in Klaveness and Lindstrom 2011; Squires et al. 1973) *H. foetidus* is considered to prefer high light intensities; it forms numerous conglomerations in the bright sunlight, while in dim light it decreases in abundance and rapidly deteriorates (Parker et al. 1973). However, its occurrence in low quantities was reported under ice (Squires et al. 1973), indicating it can tolerate low-light environments. A decreased abundance of *H. foetidus* we

observed in lit periphyton might indicate that this alga was disturbed by nocturnal artificial illumination that caused its cells to de-attach and swim downstream. The reason why this could be the case is, however, unclear. *H. foetidus* is a valuable food source for aquatic fungi, protists and aquatic insects in cold streams (see Klaveness and Lindstrom 2011), often abundant in streams worldwide, therefore its decrease under ALAN may have important implications for stream food webs.

"*Spumella*-like flagellates" are small, colorless chrysomonad flagellates that are morphologically similar or indistinguishable and therefore often grouped under this common term in ecological studies (Grossman et al. 2016). They are heterotrophic chrysophytes, widely found in freshwaters where they feed on bacteria or other protists and represent major phagotrophs in aquatic food webs and an important link between bacterial production and higher trophic levels (Boenigk et al. 2005; Grossman et al. 2016). In benthic environments they can make up 30% of biomass, although the values are usually much less (see Boenigk et al. 2005), which was also found in our study (less than 1% of total number of reads in the full data matrix). Heterotrophs in periphyton are closely linked with its autotrophic component and rely on algal exudates for food. An increased abundance of *Spumella*-like flagellates we observed under ALAN might be an indirect effect of ALAN through potentially stimulated production of exudates in algae that increased food availability for heterotrophs. An increase in light to nutrient ratio is predicted to increase bacterial growth rates (see in Hill et al. 2011); however Hölker et al. (2015) found that few months of exposure to ALAN reduced the abundance of different obligatory and facultative heterotrophic bacteria in illuminated sediments. Through which mechanisms ALAN affects heterotrophs remains unknown. The ability to assimilate particulate carbon found in many facultative heterotrophic chrysophytes and diatoms may be advantageous under limiting light conditions and provide them with competitive advantage over strictly autotrophic taxa, as it was shown for *Nitzchia* and *Navicula* (see Hill 1996; Nicholls and Wujek 2003). Jones and Rees (1994) found that ingestion rates of the facultative heterotroph *Dynobrion* in cultures were weakly influenced by irradiance, i.e. slightly higher during the dark period. ALAN may therefore directly influence feeding mechanisms of protists. How that could influence performance of facultative and strict heterotrophs remains to be determined.

The diatom *D. hyemalis* was found in higher abundancies in lit periphyton relative to the control, which indicated it might be able to use low light levels for photosynthesis and benefit from artificial nocturnal illumination. Its autecological requirements regarding light are however poorly known.

Significant effects of ALAN were observed in autumn, but not is spring. The reasons for seasonal differences in effects of ALAN may be found in different community composition of periphyton between the seasons, or physiological acclimation to seasonally different light conditions. nMDS analysis indicated that periphyton had different qualitative and quantitative composition in spring and autumn, although permutation analysis found that only 9 taxa (13 OTUs) significantly differed in relative read abundance in periphyton between the two seasons. This may indicate that physiological adaptation to seasonal variations in environmental conditions was more important determinant of sensitivity and response of periphytic taxa to ALAN in our study.

The reliability of the quantitative data obtained by next-generation sequencing methods is widely discussed in recent literature (e.g. Amend et al. 2010; Medinger et al. 2010). Many ecological studies report differences between the abundances of taxa obtained by metabarcoding methods and classical microscopical identification (e.g. Jahn et al. 2007; Zimmermann et al. 2015), These differences seem to be species-specific, but results are well correlated for the most abundant taxa and less well for the rare taxa in communities (Visco et al. 2015). The underlying biases may be related to the real biological variations (e.g. genome size and number of gene copies), technical artifacts related to primer specificity, PCR conditions and sequencing errors, or the accuracy of taxonomic assignment in the reference database. Therefore, the discrepancies in absolute numbers of reads have to be expected between the two methods and absolute numbers of reads should be interpreted with caution. However, these biases can be expected to be constant across samples, allowing for the comparison and interpretation of relative changes in read abundances between the samples (Pawlowski et al. 2016). While relying on presence-absence data overcomes these bias-related problems, in many cases the expected effects of environmental changes we aim to evaluate will be quantitative changes in communities and not the local extinction or establishment of previously absent species that could be detected in an analysis of the qualitative composition. The interpretation of quantitative community changes thus remains necessary for evaluation of ecological effects of potential stressors such as ALAN.

4.6. Conclusions

Artificial nocturnal illumination was previously found to reduce biomass accrual, increase the proportion of diatoms (in autumn) and decrease the proportion of cyanobacteria (in spring) in early periphyton stages of stream periphyton (Chapter 2). For later developmental

stages, ALAN was found to alter pigment composition of periphyton in spring and autumn (Chapter 3), indicating a decrease in diatoms/chrysophytes and an increase in cyanobacteria in spring. Here we found that ALAN altered taxonomic composition of stream periphyton in later developmental stages in autumn. ALAN significantly decreased the abundance of the dominant chrysophyte and increased the abundance of an autotrophic diatom and a heterotrophic chrysophyte relative to the control. Community diversity and presence/absence of taxa were not altered by ALAN. A weak trend in decrease of the abundance of the dominant chrysophyte taxon was observed in spring, but no effects of ALAN on spring periphyton were found either on its quantitative or qualitative composition. These results show that species-specific differences exist in the sensitivity of periphytic taxa to artificial nocturnal illumination and they confirm that seasonal variation in periphyton community composition plays an important role in shaping community response to ALAN.

Acknowledgements

The authors wish to thank the Servizio Bacini Montani, Autonomous Province of Trento, for building and maintaining the flumes; Stefan Heller (IGB), Cristina Bruno (Fondazione Edmund Mach) and Alessandro Manfrin (IGB) for help in the field. We thank Susan Mbedi and Kirsten Richter (BeGenDiv) as well as Regine Jahn, Jana Bansemer and Luis Demetrio Mora Hernández (BGBM) for their support and help in the laboratory and Calvinna Caswara (BeGenDiv) for help with OBI tools and Krona charts. This work has been carried out within the Erasmus Mundus Joint Doctorate Program SMART, funded by the EACEA, the Federal Ministry of Research and Technology, Germany (BMBF-033L038A) and the Federal Agency for Nature Conservation, Germany (3514821700).

References (Chapter 4)

Allan, J. D., and M. M. Castillo. 2007. Stream ecology: Structure and function of running waters. 2nd ed. Springer.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. **215:** 403-410.

Amend, A., K. A. Seifert, and T. D. Bruns. 2010. Quantifying microbial communities with 454 pyrosequencing: Does read abundance count? Mol. Ecol. **19:** 5555–5565.

Andersen, R. A. 2004. Biology and systematics of heterokont and haptophyte algae. Am. J. Bot. **91:** 1508-1522.

Bennion, H., C. Sayer, J. Tibby, and H. J. Carrick. 2010. Diatoms as indicators of environmental change in shallow lakes. *In* E. F. Stormer and J. P. Smol [eds.], The Diatoms: Application for the environmental and earth sciences. Cambridge University Press.

Biggs, B. J. F. 1996. Patterns in benthic algae of streams, p. 31-56. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Boenigk, J., K. Pfandl, P. Stadler, and A. Chatzinotas. 2005. High diversity of the *'Spumella*-like' flagellates: An investigation based on the SSU rRNA gene sequences of isolates from habitats located in six different geographic regions. Environ. Microbiol. **7:** 685-697.

Boyer, F., C. Mercier, A. Bonin, P. Taberlet, and E. Coissac. 2016. OBITools: A Unix-inspired software package for DNA metabarcoding. Molecular Ecology Resources **16:** 176-182.

Degnan, P. H., and H. Ochman. 2012. Illumina-based analysis of microbial community diversity. The ISME Journal **6:** 183–194.

Edgar, R. C. 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nat. Methods **10:** 996–998.

Grossman, L., C. Bock, M. Schweikert, and J. Boenigk. 2016. Small but manifold – Hidden hiversity in "*Spumella*-like flagellates". J. Eukaryot. Microbiol. **63:** 419–439.

Hill, W. R. 1996. Effects of light, p. 121-149. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Hill, W. R., B. J. Roberts, S. N. Francoeur, and S. E. Fanta. 2011. Resource synergy in stream periphyton communities. J. Ecol. **99:** 454–463.

Hölker, F., C. Wurzbacher, C. Weissenborn, M. T. Monaghan, S. I. J. Holzhauer, and K. Premke. 2015. Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. Phil. Trans. Roy. Sci. B. **370:** 20140130.

Hothorn, T., F. Bretz , and P. Westfall. 2008. Simultaneous inference in general parametric models. Biom. J. **50:** 346-363.

Jahn, R., H. Zetzsche, R. Reinhardt, and B. Gemeinholzer. 2007. Diatoms and DNA barcoding: A pilot study on an environmental sample, p. 63-68. *In* W.-H. Kusber and R. Jahn [eds.], Proceedings of the 1st central European diatom meeting. Botanic garden and Botanical museum Berlin-Dahlem, Freie Universität Berlin.

Jones, R. I., and S. Rees. 1994. Influence of temperature and light on particle ingestion by the freshwater phytoflagellate *Dinobryon*. Arch. Hydrobiol. **132:** 203–211.

Kermarrec, L. and others 2014. A next-generation sequencing approach to river biomonitoring using benthic diatoms. Freshwater Science **33:** 349-363.

Klaveness, D., and E. A. Lindstrom. 2011. *Hydrurus foetidus* (Chromista, Chrysophyceae): A large freshwater chromophyte alga in laboratory culture. Phycol. Res. **59:** 105-112.

Langdon, C. 1988. On the causes of interscpecific differences in the growth-irradiance relationship for phytoplankton. II A general review. Journal of Phytoplankton Research **10:** 1291-1312.

Lowe, R. L., and Y. Pan. 1996. Benthic algal communities as biological monitors, p. 705-739. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Medinger, R. and others 2010. Diversity in a hidden world: Potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. Mol. Ecol. **19 (Suppl. 1):** 32–40.

Nicholls, K. H., and D. E. Wujek. 2003. Chrysophycean Algae. *In* J. D. Wehr and R. G. Sheath [eds.], Freshwater algae of North America: Ecology and classification. Academic Press.

Oksanen, J. and others 2015. vegan: Community ecology package. R package version 2.4-2. https://CRAN.R-project.org/package=vegan.

Ondov, B. D., N. H. Bergman, and A. M. Phillippy. 2011. Interactive metagenomic visualization in a Web browser. BMC Bioinformatics **12**.

Parker, B. C., G. L. Samsel Jr., and G. W. Prescott. 1973. Comparison of microhabitats of macroscopic subalpine stream algae. The American Midland Naturalist **90:** 143-153.

Pawlowski, J., F. Lejzerowicz, L. Apotheloz-Perret-Gentil, J. Visco, and P. Esling. 2016. Protist metabarcoding and environmental biomonitoring: Time for change. Eur. J. Protistol. **55:** 12-25.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R. C. Team. 2015. nlme: Linear and nonlinear mixed effects models.

R Core Team. 2015. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria.

Richardson, K., J. Beardall, and J. A. Raven. 1983. Adaptation of unicellular algae to irradiance: An analysis of strategies. New Phytol. **93:** 157-191.

Smol, J. P., and E. F. Stoermer. 2010. The Diatoms: Applications for the environmental and earth sciences. Cambridge University Press.

Squires, L. E., S. R. Rushforth, and C. J. Endsley. 1973. An ecological survey of the algae of Huntington Canyon, Utah. Brigham Young University Science Bulletin, Biological Series **18:** Article 1.

Stevenson, R. J., Y. Pan, and H. Van Dam. 2010. Assessing environmental conditions in rivers and streams with diatoms. *In* E. F. Stoermer and J. P. Smol [eds.], The Diatoms: Application for the environmental and earth sciences. Cambridge University Press.

Visco, J. A., L. Apothéloz-Perret-Gentil, A. Cordonier, P. Esling, L. Pillet, and J. Pawlowski. 2015. Environmental monitoring: Inferring the diatom index from next-generation sequencing data. Environmental Science and Technology **49:** 7597−7605.

Wu, Y. 2017. Periphyton: Functions and application in environmental remediation. Elsevier.

Zimmermann, J., G. Glöckner, R. Jahn, N. Enke, and B. Gemeinholzer. 2015. Metabarcoding vs. morphological identification to assess diatom diversity in environmental studies. Molecular Ecology Resources **15:** 526–542.

Zimmermann, J., R. Jahn, and B. Gemeinholzer. 2011. Barcoding diatoms: Evaluation of the V4 subregion on the 18S rRNA gene, including new primers and protocols. Organisms Diversity & Evolution **11:** 173-192.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer.

5. Light source matters: Nocturnal low-light LED illumination decreases periphyton biomass, but high-pressure sodium does not

Maja Grubisic[1,2], Roy H.A. van Grunsven[1], Alessandro Manfrin[1,2],
Michael T. Monaghan[1], Franz Hölker[1]

[1] *Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 301/310, 12587 Berlin, Germany*
[2] *Institute of Biology, Freie Universität Berlin, Schwendenerstraße 1, 14195 Berlin, Germany*

5.1. Abstract

The increasing use of artificial light at night increases light pollution in freshwater ecosystems worldwide. Simultaneously, the spectral composition of nocturnal illumination is changing, following a current shift in outdoor lighting technologies from high-pressure sodium light (HPS) toward light emitting diodes (LED). LEDs emit broad-spectrum white light, with significant amount of blue light that is known to regulate circadian rhythms in many organisms, including primary producers. Negative effects of blue light in nocturnal illumination were demonstrated for animals and humans, but little is known about its effect on primary producers. We conducted field experiments in a lowland agricultural drainage ditch and assessed the impacts of artificial light at night by HPS and LED on primary producers in periphyton; complex benthic communities of algae, cyanobacteria and heterotrophic organisms. We compared biomass and community composition of periphyton exposed to LED or HPS nocturnal illumination of intensity comparable to light-polluted waters, with periphyton grown under a natural light regime. Pigment composition was used to infer changes in the community composition. Periphyton was collected in time series (from zero to thirteen weeks), in summer and winter for HPS lamps and only in winter for LED lamps. We found no effects of HPS light on periphyton biomass; however, LED light did decrease the biomass up to 62%. Neither light source had an effect on pigment composition. The contrasting effects on biomass between the two light sources may be explained by differences in spectral composition and in particular their blue content. Our results indicate that the type of the light source plays a role in determining impacts of artificial light at night on aquatic primary producers. Reduced biomass in the base of the food web might reflect on important ecosystem functions such as productivity and food supply for higher trophic levels.

5.2. Introduction

The current wide spread increase in nocturnal light levels due to the use of artificial light at night (ALAN) is recognized as a contributor to global environmental change (Falchi et al. 2016) and a threat to biodiversity (Hölker et al. 2010). The potential ecological effects of ALAN in illuminated ecosystems have been widely discussed (e.g. Gaston et al. 2013; Rich and Longcore 2006) and recent studies have documented effects in both terrestrial and aquatic systems (e.g. Brüning et al. 2015; Perkin et al. 2014; van Geffen et al. 2015). Along with increasing nocturnal light levels, a global shift from yellow high pressure sodium (HPS) lamps to broad-spectrum, white light-emitting diodes (LED) is taking place, resulting in a spectral shift in the nightscape (Anonymous 2012; Pawson and Bader 2014). LED lighting is often considered to be „environmentally friendly" because of its high energy-efficiency. However, reported biological effects of nocturnal LED illumination (e.g. Honnen et al. 2016; Pawson and Bader 2014; Stone et al. 2012) are raising concerns about its potential adverse ecological impacts and potentially negative consequences for human health (American Medical Association 2016; Bennie et al. 2016).

Primary producers use light as a source of energy for photosynthesis and as a source of information for synchronization of metabolic processes with their environment (Hegemann et al. 2001). Light at night can stimulate photosynthesis at times when it would not naturally occur (Aube et al. 2013). Light utilized in photosynthesis ranges from 400 to 700 nm, but blue (450 - 475 nm) and red light (630 - 675 nm) are utilized the most efficiently. Therefore a light source with strong emission in these spectral regions is more likely to stimulate photosynthesis (Aube et al. 2013). Furthermore, changes in light intensity and quality are monitored by photoreceptors and this information is 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 (400 – 500 nm) is perceived by cryptochromes and other flavin-containing receptors and regulates photosynthesis, growth and development in photosynthetic organisms (Fortunato et al. 2015). ALAN with significant emission of blue wavelengths might therefore disrupt mechanisms of internal timekeeping and affect processes regulated by circadian rhythms.

The potential impacts of ALAN on primary producers have been widely discussed for terrestrial plants (e.g. Bennie et al. 2016; Briggs 2006; Schroer and Hölker 2016). ALAN was found to cause earlier budburst and later leaf-out in trees (ffrench-Constant et al. 2016). Much less attention was given to aquatic primary producers: Poulin et al. (2014) reported that low-level ALAN by HPS (0.08 µmol m^{-2} s^{-1}, approx. 6.6 lux) affected several photophysiological

processes in cyanobacteria in laboratory cultures. ALAN was indicated to decrease the photosynthetic efficiency, but no effects on growth were observed. Hölker et al. (2015b) found that similar levels of ALAN by HPS (6.8 – 8.5 lux, approx. 0.09 µmol m^{-2} s^{-1}) significantly increased abundance of diatoms and cyanobacteria in microbial communities in sediments after one year of exposure in the field. Laboratory incubations of sampled sediments indicated that nocturnal photosynthesis was taking place under 71 lux LED, approx. 1.3 µmol m^{-2} s^{-1} (Hölker et al. 2015b). Grubisic *et al.* (Chapter 2) found that in a field study on a sub-alpine stream, ALAN by LEDs (20 lux, approx. 0.31 µmol m^{-2} s^{-1}) significantly decreased biomass and altered the proportion of diatoms in the periphyton. The effects of ALAN of different intensities reported in these laboratory and field studies were confounded by different types of applied light sources. It remained unclear which impacts of ALAN on primary producers could be expected in the real-world context and whether the spectral composition of the light source plays a role in determining those impacts. Whether ALAN would have significant effects on primary producers across different aquatic ecosystems, where periphyton communities are composed of different species with potentially different sensitivity to nocturnal illumination was not clear.

We performed experiments in a shallow, lowland agricultural ditch and assessed the effects of the two currently most widely used light sources for outdoor lighting, HPS and LED lamps, on primary producers in periphyton. Three experimental sites were set up along the ditch; one served as the ALAN treatment site (natural daylight conditions and artificial illumination at night, at a level comparable to light-polluted urban waters) with two control sites that each experienced a natural light regime. The lit site was initially equipped with HPS lamps, which were replaced by LED lamps in the consecutive year. The biomass and community composition of periphyton exposed to ALAN by HPS or LED lights were compared with those of periphyton grown under a natural light regime. We expected that yellow light from HPS lamps would have weak impact on periphyton, due to its low blue content and a mismatch of its spectral composition with the sensitivity of photosynthetic pigments. We expected that the high amount of blue light in LEDs would have a strong impact on primary producers, by potentially stimulating photosynthesis through supply of photosynthetic active radiation and by potentially disrupting circadian regulation through activation of cryptochromes. Stimulation of the nocturnal photosynthesis in autotrophs in the periphyton would result in a net biomass increase at the community level. Alternatively, the distortion of the circadian signal and nocturnal activation of the photosynthetic machinery by low-light that falls below the compensation point of photosynthesis, might come at cost (Hölker et al.

2015a; Poulin et al. 2014). If these costs outweigh the photosynthetic benefits for the primary producers in the periphyton, LED illumination might have net negative effects on the biomass of the community as a whole. Periphyton is formed by a mixture of species that differ in their sensitivity to light and photosynthetic action spectra (Jeffrey et al. 1997), therefore we expected that ALAN would differently affect individual taxa. This would result in alteration of periphyton community composition that would reflect in an altered pigment composition. To account for seasonal differences in species composition and other environmental variables, the HPS experiments were conducted in two seasons, summer and winter.

5.3. Methods

5.3.1. Sampling site and experimental design

The study was conducted in the 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 an "International Dark-Sky Reserve" by the International Dark-Sky Association (IDA). The experimental set up was installed in an agricultural area in 2012 and has been continuously running since then as part of long-term data collection program described in detail in Holzhauer et al. (2015). Two study sites were installed along a drainage ditch at 800m distance from each other. They consisted of 12 street lights in three parallel rows, 20m distant from each other, with first row 3m distant from the ditch. The ditch is approx. 5m wide, with a mean annual depth of 50 cm and is characterized by little or no water flow and thick soft sediment bottom. The riparian vegetation between the lights and the ditch was removed during the vegetation season, so that the light at the lit site could directly reach the water surface. The luminaires were equipped with 70 W high-pressure sodium 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 (19.5 ± 6.4 lux) to that of HPS lamps (17.1 ± 1.7 lux) at the water surface directly in front of the lamps. Light was measured on clear new moon nights, after astronomical twilight, with a ILT1700 underwater photometer (International Light Technologies Inc., Peabody, Massachusetts, USA). The spectral composition of the lights was measured using a compact spectrometer (specbos 1211, JETI, Jena, Germany). In the lit site, the lights were switched on at the beginning of the civil twilight and switched off at the end of civil twilight using an automatic time switch. In the control site the lights were kept off at all times. An additional

sampling site was chosen between the two sites, approx. 300 m from the lit site and located in the same ditch and 500 m from the control site (hereafter Control 1) (Fig. 14). This site served as a second control site (Control 2). Averaged minimum light levels at the two control sites were 0.002 ± 0.001 lux for Control 1 and 0.010 ± 0.010 lux for Control 2, measured on clear new moon nights.

Figure 14. Aerial view of the study area located in Westhavelland Nature Park in Brandenburg, Germany. Three experimental sites along a drainage ditch: Lit, Control 1 (C1) and Control 2 (C2). Map data provided by Google, Digital Globe, GeoBasis-DE/BKG.

Plastic DIN A4 transparent foils (polypropylene sheets with a slightly textured surface; PolyClearView, IBICO, GBC, Chicago, IL, USA) were used as substrates for the growth of periphyton. The foils are considered to support 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. The foils were cut in strips (2 cm x 15 cm) that held together as a comb. The back side of the strips was covered with the self-adhesive tape, to allow easy exclusion of periphyton that was not directly illuminated from the analysis. Foils were fixed with metal clips onto plastic frames (2 frames per site) that floated on the water surface, holding the foils in a vertical position in the upper 15 cm of the water column with tape-free side facing towards the light fixtures. For each sampling occasion four replicate strips were randomly chosen and cut from the foils at each site. The strips with visible clear paths in the periphyton cover that indicated grazing by snails were excluded from the analyses. The self-adhesive tape was removed to exclude periphyton from the back and strips were placed individually in plastic screw vials filled with pre-filtered

ditch water (Whatman GF/F glass-fibre filter, 0.7 µm nominal pore size). The vials were transported in the dark and processed in the laboratory within 5 hours.

5.3.2. Sampling procedures

The experiments under HPS lights were performed in summer and winter 2014. On July 21st and October 28th we installed the frames with the strips and collected four 1 L water samples from the middle of the ditch at all three sites. The water was used for analysis of biomass and pigment composition of the suspended phytoplankton that was available to serve as the founder community for the development of periphyton on the strips. In summer, four replicate strips were collected after one, two, three and four weeks of growth. In winter, the growth of periphyton was slower due to low temperatures and the beginning of the periphyton sampling was delayed to ensure enough biomass for analysis. In winter, the strips were collected after five, six, seven, eight and 13 weeks of growth.

On 17 July 2015, HPS lamps were replaced with LED lamps and the winter sampling was performed starting from 11 November, when we installed new frames with the strips and sampled the phytoplankton community. The strips were collected on a weekly basis from weeks one to six and again at week 13, the time point comparable to last sampled date from the winter experiment of 2014. For each sampling time, the environmental parameters (temperature, pH, dissolved oxygen and conductivity) were measured with 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 (Appendix C, Tables S4 – S6). Chemical parameters such as carbon and nutrient levels were analyzed for every sampling season according to standard chemical procedures (Krausse et al. 1983; Murphy and Riley 1962; Strickland and Parsons 1968; Wetzel and Likens 1991) (Table S7).

5.3.3. Laboratory procedures

Water samples were used to analyze the biomass and pigment composition of the phytoplankton community at all sites. After vigorous shaking, aliquots were concentrated on pre-combusted, pre-weighed 25 mm GF/F glass-fibre filters (0.7 µm nominal pore size, Whatman Ltd., Maidstone, UK) by vacuum filtration. Filters were dried at 65°C until constant mass was achieved and re-weighed for the determination of dry mass. 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). 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 Woitke et al. (1994) and Shatwell et al. (2012). Pigments were identified and quantified by their retention time and absorption spectra from standards and the literature (Jeffrey et al. 1997). Chlorophyll *a* (chl *a*) was calculated as the sum of the true chl *a* and chlorophyllids *a*, and determined as a mean of the absorption readings at 440 and 410 nm wavelength. Other pigments were determined from the absorption readings at 440 nm.

The strips with periphyton were removed from transportation vials. Periphyton was brushed with a toothbrush and rinsed with pre-filtered ditch water (Whatman GF/F glass-fibre filter) into a measuring 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 periphyton suspension was recorded and the length of the strip from which the periphyton was brushed was measured. Aliquots of the suspension were concentrated on filters for assessment of dry mass and pigment composition by HPLC, as described for phytoplankton. All manipulations were performed under dim light to avoid pigment degradation.

5.3.4. Statistical analysis

To test for effects of ALAN on biomass on phytoplankton community, we used generalized least squares linear models (GLS) (Zuur et al. 2009) as available in nlme package (Pinheiro et al. 2015) for R (Version 3.1.3, R Core Team 2015). Site ("Lit", "Control 1", "Control 2") was included as the fixed factor in the model. Post hoc comparisons were performed using t tests available in R with Benjamini-Hochberg *p* correction for multiple comparisons. GLS was also used to test effect of ALAN on periphyton biomass, including site and time (weeks) as fixed factors in the model. Time series data was tested using package tseries for R (Trapletti and Hornik 2017). To account for temporal correlation, the model included in the model 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 levels of fixed factors (site or time) were used as variance covariates (Zuur et al. 2009). Pairwise comparisons were performed using multcomp package for R (Hothorn et al. 2008) with Benjamini-Hochberg *p* correction. Summer and winter communities were analyzed separately, as the periphyton community composition was expected to differ between the seasons.

Pigment composition was used as a composite indicator of periphyton community composition. Pigment concentrations were normalized to chl *a*, z-standardized and analyzed

using principal component analysis (PCA) using 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 were statistically tested using the same GLS model as for biomass, i.e. including site and time as fixed factors and an auto-correlation structure in the model. 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.

5.4. Results

5.4.1. Phytoplankton community under HPS lighting

In summer, the biomass of the phytoplankton was different between the experimental sites (GLS: site effect $F_{2, 25} = 25.25$, $p < 0.001$). The biomass was significantly higher at Control 1 site (15.21 ± 4.21, mean and SD) compared with both Lit site (4.06 ± 1.27, $p < 0.0001$) and at the Control 2 site (2.41 ± 1.23, $p < 0.0001$). There were no differences in biomass between the lit site and the Control 2 ($p = 0.06$).

Three chlorophyll pigments and seven carotenoid pigments were identified in the phytoplankton samples in summer (Appendix C, Table S8). A PCA based on relative pigment concentrations (Fig. S4a) showed that 52% of the variance could be explained by the first principal component (PC1). PC1 clearly separated the three sites and was strongly correlated with lutein (Pearson's $r = - 0.99$), chl *b* ($r = - 0.93$) and neoxanthin ($r = - 0.93$). PC1 scores indicated that phytoplankton communities from all three sites were significantly different from each other in pigment composition (Fig. S5a, GLS: site effect $F_{1,2} = 143.26$, $p < 0.0001$. *t* test: Lit to Control 1 $p = 0.0001$, Lit to Control 2: $p < 0.0001$, Control 1 to Control 2 $p < 0.0001$).

In winter, we observed similar pattern in phytoplankton biomass as for summer. The biomass differed between the sites (GLS: site effect $F_{1,2} = 7.48$, $p = 0.01$) and was significantly higher at the Control 1 site (67.48 ± 4.69, mean and SD) then both the Lit site (39.61 ± 8.20, $p = 0.01$) and the Control 2 site (46.93 ± 12.17, $p = 0.04$). The biomass at the Lit site did not differ from the Control 2 ($p = 0.29$).

Six pigments were identified in phytoplankton in winter (Table S8). PC1 accounted for 52% of the variance in pigment composition and clearly separated Control 1 from the two other sites (Fig. S4b). PC1 was strongly correlated with diadinoxanthin (Pearson's $r = - 0.96$)

and lutein (r = - 0.99). PC1 scores were significantly affected by sites (Fig. S5b, GLS: site effect $F_{1,2}$ = 44.74, $p < 0.001$), indicating that phytoplankton community at Control 1 significantly differed from both the Lit site ($p < 0.001$) and the Control 2 ($p < 0.001$). There were no differences between the Lit site and the Control 2 site ($p = 0.55$).

In summary, the spatially closer sites (Lit and Control 2) clearly differed from the Control 1 while ALAN had no significant effect on phytoplankton biomass. Except for the pigment composition in summer, the phytoplankton communities at the Lit and Control 2 site did not differ from each other.

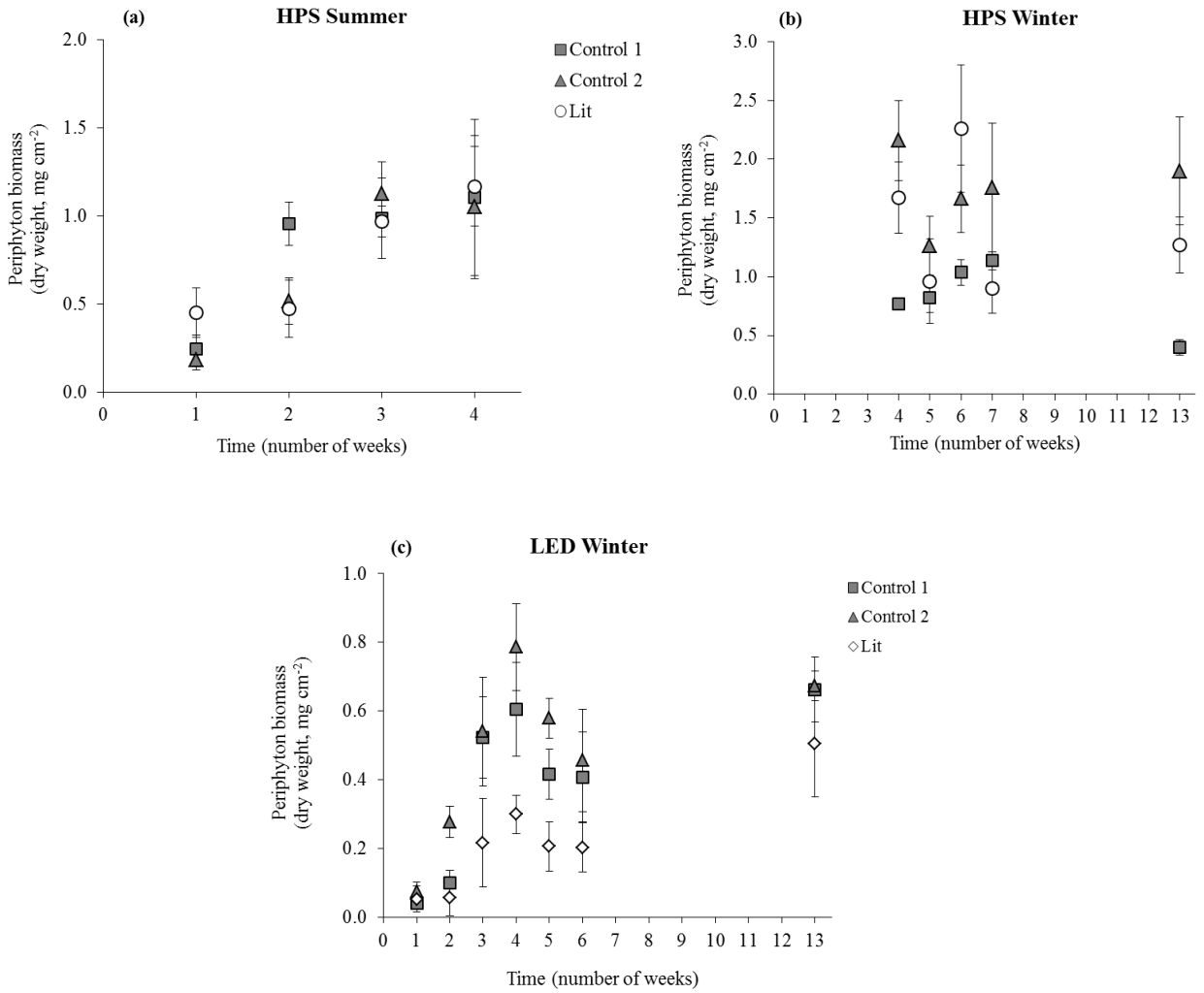

Figure 15. The biomass of periphyton (dry mass, mg cm^{-2}, mean and SD) developed at the two control sites (Control 1 and Control 2) and at the lit site, under high-pressure sodium lamps (HPS) (a, b) or LED lamps (c) in summer (a, n = 48) and in winter (b, n = 60; c, n = 84).

5.4.2. Periphyton under HPS lighting

In summer, the biomass of periphyton that developed on the substrates increased with time at all three sites (Fig. 15a. GLS: time effect $F_{1,1} = 76.53$, $p < 0.0001$). There were no differences in biomass between the sites (GLS: site effect $F_{1,2} = 0.64$, $p = 0.53$) and no significant interaction with time (GLS: site x time effect $F_{1,2} = 0.28$, $p = 0.75$).

The same pigments that were identified in the summer phytoplankton community were also identified in the periphyton in summer (Table S9). PC1 accounted for 54% of variance in the pigment composition (Fig. 16a) and clearly separated the Control 1 site from the two others. PC1 was correlated with violaxanthin (Pearson's $r = - 0.93$), lutein ($r = - 0.92$), chl *b* ($r = - 0.87$) and neoxanthin ($r = - 0.87$). PC1 scores indicated that periphyton significantly differed in pigment composition at all sites (Fig. S6a, GLS: site effect $F_{1,2} = 47.95$, $p < 0.0001$. *t* test: Lit to Control 1: $p < 0.0001$, Lit to Control 2: $p = 0.006$, Control 1 to Control 2: $p < 0.0001$). Changes were significant over time (time effect $F_{1,1} = 5.53$, $p = 0.02$) and there was no significant interaction (site x time effect $F_{1,2} = 2.59$, $p = 0.09$).

In winter, the biomass of periphyton changed non-linearly and significantly over time (Fig. 15b, GLS: time effect $F_{1,1} = 20.15$, $p < 0.001$) and across sites (GLS: site effect $F_{1,2} = 18.33$, $p < 0.0001$). The biomass at the Lit site (1.41 ± 0.60, mean and SD) did not differ from biomass at the Control 2 site (1.75 ± 0.46, *t* test: $p = 0.22$) and both were significantly higher than that of Control 1 (0.83 ± 0.27, *t* test: Lit to Control 1: $p < 0.0001$, Control 1 to Control 2: $p < 0.0001$). The interaction between site and time was not significant (GLS: site x time $F_{1,2} = 2.19$, $p = 0.12$).

Ten pigments were identified in the periphyton in winter (Table S10). PC1 accounted for 50% of the variance in pigment composition (Fig. 16b) and was correlated with diadinoxanthin (Pearson's $r = - 0.94$) and lutein ($r = - 0.86$). Time-induced variation in pigment composition is visible along PC1, whose scores indicated that periphyton significantly changed in pigment composition over time (Fig. S6b, GLS: time effect $F_{1,1} = 15.22$, $p = 0.0003$) at all experimental sites (GLS: site effect $F_{1,2} = 5.93$, $p = 0.005$). A significant interaction (GLS: site x time effect $F_{1,2} = 6.55$, $p = 0.003$) indicated that periphyton pigment composition has changed differently at all sites over time.

In summary, the differences in biomass and pigment composition found for periphyton did not reflect the spatial patterns that were observed for phytoplankton communities. No clear patterns were observed related to the nocturnal light conditions.

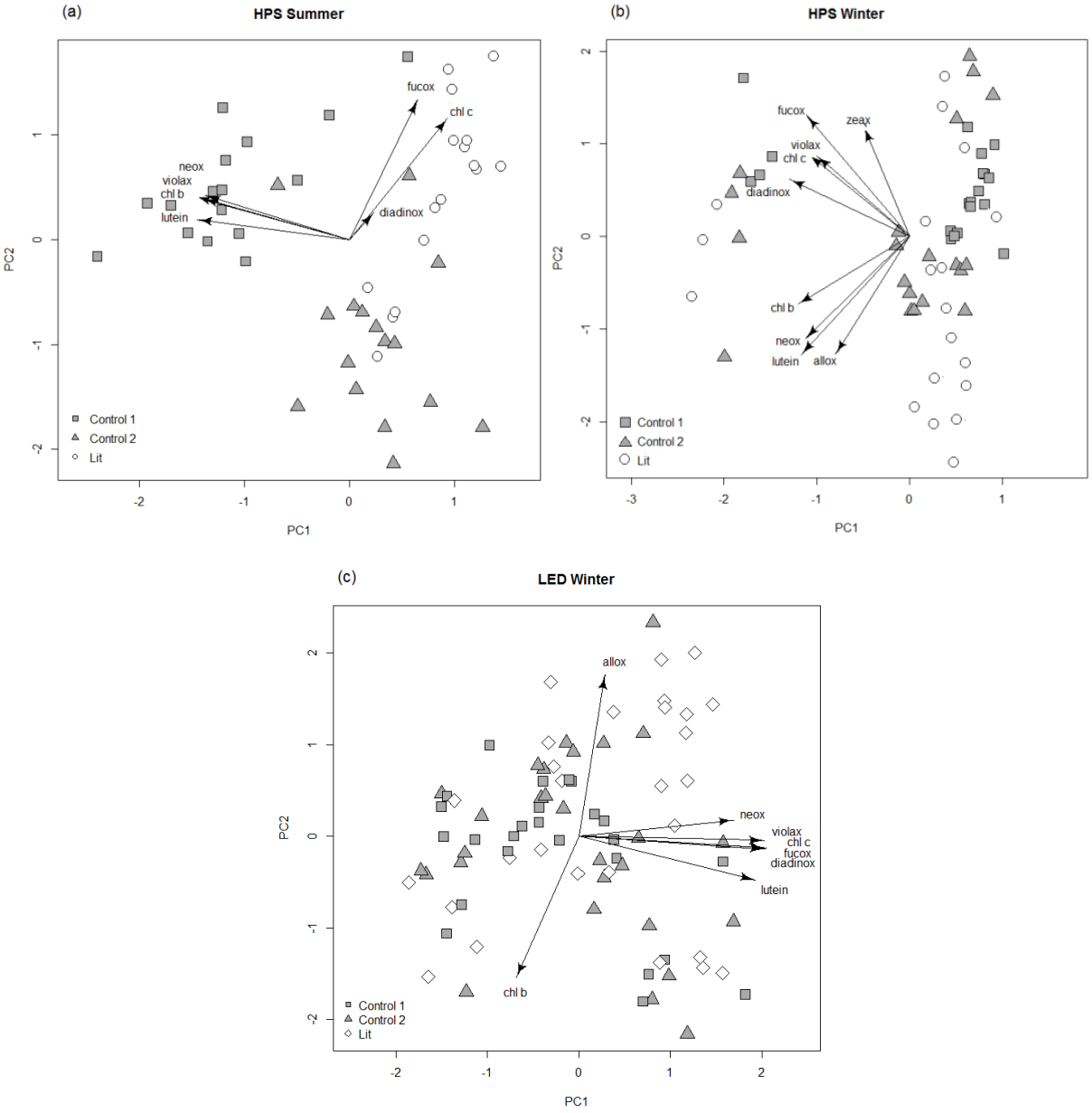

Figure 16. Correlation biplots of principal component analysis (PCA) based on relative pigment concentrations of periphyton, normalized to chlorophyll a, developed at the two control sites (Control 1 and Control 2) and at the Lit site, under high-pressure sodium lamps (HPS) (a, b) or LED lamps (c) in summer (a, n = 48) and in winter (b, n = 60; c, n = 84). Planes of the first two PC axes that explain 76% (a), 73% (b) and 89% (c) of variation in the data. The pigments included in the analysis are: chlorophyll *b* (chl b), chlorophyll *c* (chl c), fucoxantin (fucox), violaxanthin (violax), diadinoxanthin (diadinox), neoxanthin (neox), zeaxanthin (zeax),

alloxanthin (allox) and lutein. Arrows show correlation between original pigment variables with PC axes and all indicate significant relationship ($p < 0.0001$).

5.4.3. Phytoplankton community under LED lighting

The experiment performed in the following winter of 2015, after the lights were replaced with LEDs, showed that the biomass of the phytoplankton significantly differed between the sites (GLS: site effect $F_{1,2} = 62.48$, $p < 0.0001$). All sites differed from each other (mean and SD: Lit 4.06 ± 1.27, Control 1 15.21 ± 4.21, Control 2 2.41 ± 1.23. *t* test: Lit to Control 1: $p < 0.001$, Lit to Control 2: $p < 0.001$, Control 1 to Control 2: $p = 0.002$).

Three chlorophyll pigments and six carotenoid pigments were identified in the phytoplankton (Table S8). PC1 accounted for 50% of variation in the pigment composition (Fig. S4c) and strongly correlated with violaxanthin (Pearson's $r = -0.92$) and alloxanthin ($r = -0.97$). Site-driven variation in pigment composition is visible along PC1, whose scores indicated that phytoplankton communities clearly differed in pigment composition between the sites (Fig. S5c, GLS: site effect $F_{1,2} = 13.56$, $p = 0.002$). Phytoplankton significantly differed from each other at all sites (*t* test: Lit to Control 1 $p = 0.03$, Lit to Control 2 $p = 0.002$, Control 1 to Control 2 $p = 0.03$).

5.4.4. Periphyton under LED lighting

The biomass of periphyton was significantly affected by site (Fig. 15c, GLS: site effect $F_{1,2} = 15.02$, $p < 0.0001$), but not time (GLS: time effect $F_{1,1} = 2.52$, $p = 0.12$). Starting from three weeks of growth until the end of the experiment (13 weeks), the biomass at the Lit site was significantly lower compared to the Control 1 (*t* test: $p < 0.0001$) and the Control 2 (*t* test: $p < 0.0001$). The two control sites did not significantly differ from each other (*t* test: $p = 0.06$). The interaction between site and time was not significant (GLS: site x time effect $F_{1,2} = 1.08$, $p = 0.34$).

The same ten pigments that were identified in the phytoplankton communities were identified in the periphyton (Table S11). PC1 accounted for 68% of the variance in pigment composition (Fig. 16c) and strongly 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 between the sites (Fig. S6c, GLS: site effect $F_{1,2} = 15.18$, $p < 0.0001$). Pairwise comparisons found significant differences between all three sites (*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 $F_{1,1} = 2.49$, $p = 0.12$) and no significant interaction between site and time (GLS: site x time effect $F_{1,2} = 0.38$, $p = 0.69$).

In summary, the observed differences for phytoplankton in both biomass and pigment composition did not directly reflect the differences in light regime, as the communities differed from each other at all experimental sites. However, the biomass of periphyton that developed under ALAN by LED was significantly reduced relative to the two control sites.

5.5. Discussion

We found that periphyton community exposed to low-level LED light at night had lower biomass compared to periphyton that experienced natural day-night light regime in a lowland agricultural ditch in winter. Nocturnal illumination by HPS light of comparable intensity (around 20 lux) had no effect on periphyton biomass in either winter or summer. Neither light source significantly affected periphyton community composition, as indicated by the relative pigment composition. Our results confirm that LED light at night can decrease the biomass of freshwater periphyton and show that this decrease is significant in a lowland freshwater system. The type of the light source, likely its spectral composition and in particular its blue light component, played a significant role in determining biological impacts of ALAN on periphyton biomass.

To contribute significantly to photosynthesis, ALAN has to be of sufficient intensity, providing enough energy to reach the photosynthetic compensation point and activate photosynthetic machinery. Additionally, light needs to be of certain quality and contain wavelengths that match the sensitivity of the photosynthetic pigments. Although photosynthesis theoretically 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 with the maximum light of a full moon on a clear night (0.005 µmol m^{-2} s^{-1}, i.e. below 0.3 lux) (Raven and Cockell 2006), the stimulating effects of ALAN on photosynthesis are considered to be very limited in the real-world context. Empirical evidence suggests that minimum light for phototrophic growth among microalgae is below 1 µmol m^{-2} s^{-1} (approx. 50 lux) (Richardson et al. 1983) but the thresholds are not well known and also not known for artificial light. Poulin et al. (2014) showed that ALAN of 0.08 µmol m^{-2} s^{-1} (approx. 6.6 lux, HPS) did not affect growth rate of cyanobacteria *Microcycstis aeruginosa*, but did affect several photophysiological variables. However, microbial communities from sediments that were exposed to low-level ALAN (6.8 – 8.5 lux, approx. 0.09 µmol m^{-2} s^{-1}, HPS) for one year showed positive nocturnal net production when incubated at higher ALAN level of 71 lux (approx. 1.3 µmol m^{-2} s^{-1}, LED) in the laboratory (Hölker et al. 2015b).

Although the light intensity applied in our experiment (approx. 20 lux, 0.31 µmol m^{-2} s^{-1}) was likely high enough to stimulate nocturnal photosynthesis, exposure to LED at night resulted in decreased periphyton biomass on a community level. Therefore the increase in photosynthesis by LEDs had to be outweighed by the negative effects of LED light at night, resulting in decreased community performance and decreased biomass production.

ALAN replaces the dark phase of 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. Algae and cyanobacteria are sensitive to changes in light intensity and spectral quality (Fortunato et al. 2015; Mullineaux 2001) and can detect light of intensity far below threshold for photosynthesis, including moonlight (approx. 0.1 lux) (Bünning and Moser 1969) via photoreceptors. Since algae are normally exposed to cycles of light and dark in their natural environment, it is generally assumed that dark periods are necessary for their optimal growth (Carvalho et al. 2011). In many microalgal species DNA replication and cell division occur in darkness and some phases of cell division cycle might be sensitive to light (Edmunds 1988). Furthermore, natural darkness is considered to be crucial for stress recovery and repair in plants (Gaston et al. 2013). The interruption of a dark phase in a light/dark cycle by impulses of bright light was reported to negatively affect growth of several diatom species (Gibson and Fitzsimons 1991; Gibson and Fitzsimons 1992; Zevenboom and Mur 1984); however, the differences were reported to exist between the investigated species. Light/dark cycles are important regulators of timing of cell cycle, embryogenesis, gametogenesis and many cellular processes such as synthesis of various cellular components and chloroplast differentiation (Hegemann et al. 2001). In particular, blue light perceived by cryptochromes and other flavin-containing photoreceptors synchronizes the circadian clock with external light-dark cycles, which is crucial for photosynthesis, growth and survival of algae (Dodd et al. 2005) as well as DNA repair, pigment biosynthesis and chloroplast development (Fortunato et al. 2015; Hegemann et al. 2001). In plants and moss, changes in light regime have been shown to cause circadian stress, characterized by altered expression of clock genes and reduced photosynthetic efficiency (Okada et al. 2009). The alteration of a natural light regime by ALAN is likely to cause a disruption in activity of clock genes in algae and cyanobacteria as well.

The pronounced blue peak in emission spectra of LEDs, to which algae and cyanobacteria are highly sensitive (Fig. 17), might be therefore responsible for observed negative effects of LED on periphyton. Blue light in nocturnal illumination might interfere with the entrainment of the circadian clock in primary producers and thereby regulated physiological and

developmental processes, decreasing the net performance of periphyton communities and resulting in reduced biomass production. Light emitted by HPS lamps contains very little blue and is rich in yellow wavelengths that do not disrupt entrainment of circadian clock and are not efficiently absorbed by photosynthetic pigments. It is likely that due to the mismatch between spectral composition of light emitted by HPS and sensitivity of photosynthetic pigments and photoreceptors (Fig. 17), nocturnal illumination by HPS had no significant effect on periphyton communities.

Apart from light, nutrients and grazers are also strong determinants of periphyton biomass (Stevenson 1996). The levels of nutrients varied between the experimental sites across all seasons (Table S7) but there was no nutrient limitation at the lit site that could explain the observed biomass reduction under LED lights. The grazers that were present in the system belonged to zooplankton, free-swimming invertebrates such as mayflies, snails and fish (pers. obs.). Strips that were grazed by snails were excluded from the analyses. The consumption by free swimming grazers in winter was considered to be unimportant based on previous observations, although the grazing activity was not explicitly assessed in this study.

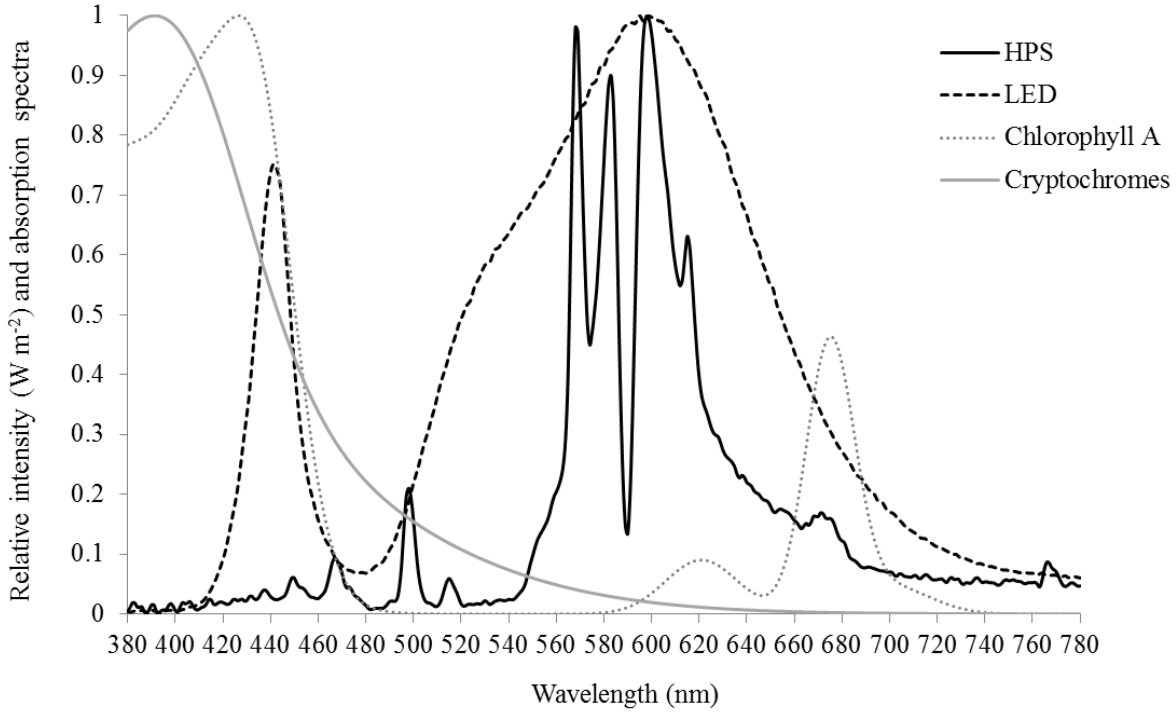

Figure 17. Spectral composition of the two light sources used in the study (high-pressure sodium, HPS and light-emitting diodes, LED), spectral absorption curve of chlorophyll *a* (based on Lohrenz et al. 2003) and spectral sensitivity of cryptochromes (based on Malhotra et al. 1995).

The illumination in the lit site started one year before the beginning of our study; therefore the phytoplankton communities at experimental sites were already exposed to the artificial nocturnal illumination. We characterized the biomass and pigment composition of phytoplankton communities to assess whether these potential founder communities were already different at the lit site compared to the controls, as these differences may be attributed to the pre-exposure to ALAN and could potentially translate to periphyton communities developed on the strips. The biomass and pigment composition of phytoplankton could not be directly linked to the light regime as the initial biomass was different at all sites for the LED winter experiment. This indicated that pre-exposure to ALAN did not systematically affect phytoplankton. The lowest biomass of phytoplankton was recorded at the Control 2 site (41% lower than at the Lit site and 84% lower than at the Control 1), while the biomass of periphyton was significantly lower at the Lit site compared to the two controls. We thus believe that the decrease in the periphyton biomass observed under LEDs was a true effect of artificial nocturnal illumination rather than the effect of the founder communities.

5.6. Conclusions

Nocturnal LED illumination was previously reported to decrease the biomass of periphyton in a stream-side flume system on a sub-alpine stream (Chapter 2). Here, we found that the biomass decrease also appears under LED light at night in a strongly contrasting environmental context, a lowland freshwater system, whose periphyton communities are characterized by different taxa with different ecological characteristics. Our results suggest that a reduced growth may be a general response of periphyton to nocturnal LED illumination. The response of periphyton to artificial light at night, however, is strongly influenced by the light source, likely on its spectral composition, in particular its blue content. Periphyton is an important ecosystem component in streams, ponds, wetlands and clear shallow lakes where it forms the base of the food web and participates in biochemical cycling. The decreased biomass under ALAN may thus potentially decrease nutrient turnover and food supply for higher trophic levels, lowering production in the illuminated ecosystems. The current increase in use of nocturnal illumination and the concurrent worldwide shift from HPS to LED lights in outdoor lighting are likely to increase these effects on a landscape scale (Davies et al. 2013; Schroer and Hölker 2016). A proof that the switch to LED light degrades ecosystem functioning would have major ramifications for ecological conservation, as well as for lighting policy and urban planning.

Acknowledgements

The experimental sites were installed within the interdisciplinary research project Verlust der Nacht (Loss of the Night) which was supported by the Federal Ministry of Research and Technology, Germany (BMBF-033L038A). This work was partially carried out within the Erasmus Mundus Joint Doctorate Program SMART, funded by the Education, Audiovisual and Culture Executive Agency of the European Commission. Funding was also provided by the Federal Agency for Nature Conservation, Germany (3514821700). The authors wish to thank Stefan Heller (IGB) and Sibylle Schroer (IGB) for help with practical organization, Emily Mattig for help with data collection in winter 2014, Barbara Stein (IGB) for performing HPLC analysis, Gabriel Singer for helpful discussions and the "Monday meeting group" (IGB) for suggestions on the manuscript.

References (Chapter 5)

Anonymous. 2012. Lighting the way: Perspectives on the global lighting market. McKinsey and Company.

Association, A. M. 2016. Human and environmental effects of light emitting diode (LED) community lighting. Report of the council on science and public health. Chicago (IL): American Medical Association; CSAPH Report 2-A-16**:** 8.

Aube, M., J. Roby, and M. Kocifaj. 2013. Evaluating potential spectral impacts of various artificial lights on melatonin suppression, photosynthesis, and star visibility. Plos One **8**.

Bennie, J., T. W. Davies, D. Cruse, and K. J. Gaston. 2016. Ecological effects of artificial light at night on wild plants. J. Ecol. **104:** 611–620.

Briggs, W. R. 2006. Physiology of plant responses to artificial lighting. *In* C. Rich and T. Longcore [eds.], Ecological consequences of artificial night lighting. Island Press.

Brothers, S. M., S. Hilt, S. Meyer, and J. Kohler. 2013. Plant community structure determines primary productivity in shallow, eutrophic lakes. Freshwat. Biol. **58:** 2264-2276.

Brüning, A., F. Hölker, S. Franke, T. Preuer, and W. Kloas. 2015. Spotlight on fish: Light pollution affects circadian rhythms of European perch but does not cause stress. Sci. Total Environ. **511:** 516-522.

Bünning, E., and I. Moser. 1969. Interference of moonlight with the photoperiodic measurement of time by plants, and their adaptive reaction. Proc. Natl. Acad. Sci. USA **62:** 1018-1022.

Carvalho, A. P., S. O. Silva, J. M. Baptista, and F. X. Malcata. 2011. Light requirements in microalgal photobioreactors: An overview of biophotonic aspects. Appl. Microbiol. Biotechnol. **89:** 1275-1288.

Davies, T. W., J. Bennie, R. Inger, N. H. De Ibarra, and K. J. Gaston. 2013. Artificial light pollution: Are shifting spectral signatures changing the balance of species interactions? Global Change Biol. **19:** 1417-1423.

Dodd, A. N. and others 2005. Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science **309:** 630-633.

Falchi, F. and others 2016. The new World Atlas of artificial night sky brightness. Sci. Adv. **2:** e1600377.

ffrench-Constant, R. H. and others 2016. Light pollution is associated with earlier tree budburst across the United Kingdom. Proc. R. Soc. Lond., Ser. B: Biol. Sci. **283**.

Fortunato, A. E., R. Annunziata, M. Jaubert, J.-P. Bouly, and A. Falciatore. 2015. Dealing with light: The widespread and multitasking cryptochrome/photolyase family in photosynthetic organisms. J. Plant Physiol. **172:** 42-54.

Gaston, K. J., J. Bennie, T. W. Davies, and J. Hopkins. 2013. The ecological impacts of nighttime light pollution: A mechanistic appraisal. Biol. Rev. **88:** 912-927.

Gibson, C. E., and A. G. Fitzsimons. 1991. Light break in the dark period depresses the growth rate of a freshwater planktonic diatom. Diatom Res. **6:** 15-20.

Gibson, C. E., and A. G. Fitzsimons. 1992. The effect of an interrupted dark period on the growth rate of some marine and freshwater planctonic diatoms. Diatom Res. **7:** 199-201.

Hegemann, P., M. Fuhrmann, and S. Kateriya. 2001. Algal sensory photoreceptors. J. Phycol. **37:** 668–676.

Hölker, F., C. Wolter, E. K. Perkin, and K. Tockner. 2010. Light pollution as a biodiversity threat. Trends Ecol. Evol. **25:** 681-682.

Hölker, F., C. Wurzbacher, C. Weissenborn, M. T. Monaghan, S. I. J. Holzhauer, and K. Premke. 2015a. Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. Phil. Trans. Roy. Sci. B. **370:** 20140130.

Holzhauer, S. I. J. and others 2015. Out of the dark: Establishing a large-scale field experiment to assess the effects of artificial light at night on species and food webs. Sustainability **7:** 15593-15616.

Honnen, A.-C., P. R. Johnston, and M. T. Monaghan. 2016. Sex-specific gene expression in the mosquito Culex pipiens f. molestus in response to artificial light at night. Bmc Genomics **17**.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal **50:** 346-363.

International Dark Sky Association (IDA). International Dark Sky Reserves: Westhavelland (Germany). Available online: http://darksky.org/idsp/reserves/westhavelland/

Jeffrey, S. W., R. F. C. Mantoura, and S. W. Wright (eds.). 1997. Phytoplankton pigments in oceanography: Guidelines to modern methods. UNESCO Publishing, France.

Krausse, G. L., C. L. Schelske, and C. O. Davis. 1983. Comparison of three wet-alkaline methods of digestion of biogenic silica in water. Freshwat. Biol. **13**.

Lohrenz, S. E., A. D. Weidemann, and M. Tuel. 2003. Phytoplankton spectral absorption as influenced by community size structure and pigment composition. J. Plankton Res. **25:** 35-61.

Malhotra, K., S.-T. Kim, A. Batschauer, L. Dawut, and A. Sancar. 1995. Putative blue-light photoreceptors from *Arabidopsis thaliana* and *Sinapis alba* with a high degree of sequence homology to DNA photolyase contain the two photolyase cofactors but lack DNA repair activity. Biochemistry **34:** 6892-6899.

Mullineaux, C. W. 2001. How do cyanobacteria sense and respond to light? Mol. Microbiol. **41:** 965-971.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta **27:** 31-36.

Okada, R., S. Kondo, S. B. Satbhai, N. Yamaguchi, M. Tsukuda, and S. Aoki. 2009. Functional characterization of CCA1/LHY homolog genes, PpCCA1a and PpCCA1b, in the moss *Physcomitrella patens*. Plant J. **60:** 551-563.

Oksanen, J. and others 2015. vegan: Community ecology package. R package version 2.4-2. https://CRAN.R-project.org/package=vegan.

Pawson, S. M., and M. K. F. Bader. 2014. LED lighting increases the ecological impact of light pollution irrespective of color temperature. Ecol. Appl. **24:** 1561-1568.

Perkin, E. K., F. Hölker, and K. Tockner. 2014. The effects of artificial lighting on adult aquatic and terrestrial insects. Freshwat. Biol. **59:** 368-377.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R. C. Team. 2015. nlme: Linear and nonlinear mixed effects models.

Poulin, C., F. Bruyant, M.-H. Laprise, A. M. Cockshutt, J. M.-R. Vandenhecke, and Y. Huot. 2014. The impact of light pollution on diel changes in the photophysiology of *Microcystis aeruginosa*. J. Plankton Res. **36:** 286-291.

R Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raven, J. A., and C. S. Cockell. 2006. Influence on photosynthesis of starlight, moonlight, planetlight, and light pollution (reflections on photosynthetically active radiation in the universe). Astrobiology **6:** 668-675.

Rich, C., and T. Longcore. 2006. Ecological consequences of artificial night lighting. Island Press.

Richardson, K., J. Beardall, and J. A. Raven. 1983. Adaptation of unicellular algae to irradiance: An analysis of strategies. New Phytol. **93:** 157-191.

Schroer, S., and F. Hölker. 2016. Impact of lighting on flora and fauna, p. 1-33. *In* R. Karlicek, C.-C. Sun, G. Zissis and R. Ma [eds.], Handbook of advanced lighting technology. Springer International Publishing.

Shatwell, T., A. Nicklisch, and J. Köhler. 2012. Temperature and photoperiod effects on phytoplankton growing under simulated mixed layer light fluctuations. Limnol. Oceanogr. **57:** 541-553.

Soetaert, K. 2014. shape: Functions for plotting graphical shapes, colors.

Stevenson, R. J. 1996. An introduction to algal ecology in freshwater benthic habitats. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Stone, E. L., G. Jones, and S. Harris. 2012. Conserving energy at a cost to biodiversity? Impacts of LED lighting on bats. Global Change Biol. **18:** 2458-2465.

Strickland, J. D. H., and T. R. Parsons. 1968. A practical handbook of seawater analysis. Fisheries Research Board of Canada.

Trapletti, A., and K. Hornik. 2017. tseries: Time series analysis and computational finance. R package version 0.10-37.

van Geffen, K. G. and others 2015. Artificial light at night inhibits mating in a Geometrid moth. Insect Conserv. Diver. **8:** 282-287.

Wetzel, R. G., and G. E. Likens. 1991. Limnological analysis, 2nd edn. . Springer.

Woitke, P., C.-D. Martin, S. Nicklisch, and J.-G. Kohl. 1994. HPLC determination of lipophilic photosynthetic pigments in algal cultures and lake water samples using a non-endcapped C18-RP-column. Fresenius J. Anal. Chem. **348:** 762-768.

Zevenboom, W., and L. R. Mur. 1984. Growth and photosynthetic response of the cyanobacterium Microcystis aeruginosa in relation to photoperiodicity and irradiance. Arch. Microbiol. **139:** 232-239.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer.

6. General discussion

Our knowledge about biological and ecological impacts of artificial nocturnal illumination is accumulating at an increasing rate. It is clear that the disruption of the natural light regime has multiple consequences for physiology, behaviour and performance of individual organisms (Gaston et al. 2015), but also affects communities, populations and ecosystems (e.g. Altermatt and Ebert 2016; Davies et al. 2015). Since Perkin et al. (2011) identified existing knowledge gap regarding freshwater ecosystems, we are increasingly gaining information on effects of artificial light at night on aquatic microorganisms (Hölker et al. 2015; Poulin et al. 2014), invertebrates (e.g. Meyer and Sullivan 2013; Perkin et al. 2014a; Perkin et al. 2014b) and fish (e.g. Becker et al. 2013; Brüning et al. 2015; Riley et al. 2012). This thesis investigated the effects of ALAN on communities of benthic primary producers and increases our understanding of its impacts on the base of aquatic food webs. These can have important implications for ecosystem functions in freshwaters exposed to artificial nocturnal illumination.

6.1. Key research findings

In this thesis I presented evidence that artificial light at night (ALAN), as found in urban and sub-urban areas, influences benthic communities of primary producers in freshwater ecosystems by reducing the biomass of periphyton in different ecological contexts and altering the proportions of several autotrophic groups in periphyton communities. These effects were found to be influenced by: a) the type of the light source, with LED resulting in strong decrease in biomass in contrast to high-pressure sodium light that had no effect; b) the colonization stage of the community, where periphyton in early stages (up to three weeks) was found to be more sensitive to ALAN relative to later colonization stages (three to six weeks); and c) season, that presumably played a significant role in determining periphyton species composition and physiological adaptation to seasonal variation in light, thus determining the sensitivity of periphytic taxa to low-level nocturnal illumination. The results presented in this thesis are summarized in Table 5.

Table 5. Overview of the effects of artificial light at night found in this thesis, using different methods (n.e. stands for no effects, - for not assessed with the given method, NA for data not available). All effects are based on significant differences ($p < 0.05$). A "+" indicates a near-significant effect ($p = 0.068$).

					Biomass		**Community composition**	
Freshwater system	Light source	Method	Level	Developmental stage	Spring	Autumn	Spring	Autumn
Sub-alpine stream	LED	*In-situ flurometry*	Community	Early	↓	↓	↓ cyanobacteria	↑ diatoms
				Late	n.e.	n.e.	n.e.	n.e.
		High pressure liquid chromatography (HPLC)	Community	Late	n.e.	n.e.	↓ diatoms /chrysophytes ↑ cyanobacteria ↑ diadinoxanthin	↓ diatoxanthin
		DNA metabarcoding	Community	Late	-	-	n.e.	change in taxa abundancies
			Individual	Late	-	-	↓ *Hydrurus foetidus*+ (chrysophytes)	↓ *Hydrurus foetidus* ↑ *Spumella*-like flagellate (chrysophytes) ↑ *Diatoma hyemalis* (diatoms)
					Summer	Winter	Summer	Winter
Lowland agricultural ditch	LED	*Dry mass*	Community	Late	NA	↓	-	-
		HPLC	Community	Late	NA	↓	NA	n.e.
	HPS	*Dry mass*	Community	Late	n.e.	n.e.	-	-
		HPLC	Community	Late	n.e.	n.e.	n.e.	n.e.

In **Chapter 2** I found that exposure of stream periphyton in early developmental stages (up to three weeks) to LED at night decreased the biomass of periphyton (in spring and autumn), increased the relative abundance of the dominant group, the diatoms (in autumn) and decreased the relative abundance of cyanobacteria (in spring) after three weeks. In contrast, the exposure of periphyton to ALAN at later stages of community development (three to six weeks) did not have an effect on biomass or community composition, as assessed using *in-situ* fluorometry (the BenthoTorch), a relatively new method for rapid assessment of periphyton biomass and community composition. The observed biomass decrease in lit periphyton was surprising, as it contradicted my hypothesis that ALAN would stimulate nocturnal photosynthesis and enhance periphyton biomass accrual. A major factor that could have contributed to biomass loss is grazing by herbivores that were present in the system. However, the exposed position of the tiles and the known suppression of insects' movement under nocturnal illumination at intensities far below that applied in our experiment, made it unlikely that grazers were strong determinant of periphyton biomass. Assessment of macroinvertebrate communities by Manfrin et al. (in prep.) confirmed that there were no differences in macroinvertebrate communities between the treatments and that grazing was not increased under artificial light at night. Thus, the net adverse effect of ALAN on periphyton biomass that we observed had to result from another mechanism than photosynthesis or grazing. Measurements at the physiological level were beyond the scope of my study; but the literature suggests several non-exclusive alternative explanations for the observed decrease in biomass:

a) Artificial nocturnal illumination of intensity that falls below the compensation point of photosynthesis might be energetically costly for primary producers, because it may keep the photosynthetic machinery active at night but does not provide enough energy for photosynthetic production (Hölker et al. 2015; Poulin et al. 2014);

b) Artificial light at night replaces darkness in natural day/night i.e. light/dark cycles, to which primary producers are normally exposed in the nature and which is considered to be necessary for several physiological processes, including DNA repair and stress recovery (Gaston et al. 2013);

c) The disruption of natural light/dark cycles by ALAN may be detected by photoreceptors that are involved in circadian regulation e.g. chryptochromes and disrupt numerous internal processes regulated by circadian clock (Gaston et al. 2013; Hegemann et al. 2001).

The threshold values of light intensity necessary for protosynthetic production are not accurately determined in the literature, but are considered to lie between 0.01 µmol m^{-2} s^{-1},

approx. 0.5 lux (based on theoretical calculations) (Raven and Cockell 2006) and 1 µmol m^{-2} s^{-1}, approx. 50 lux (based on laboratory observations) (Richardson et al. 1983). The light intensity applied in our experiment (0.31 µmol m^{-2} s^{-1}, approx. 20 lux) might have stimulated photosynthesis, but we did not find an increase in biomass to confirm this. Photoreceptors are, however, extremely sensitive to changes in light intensity and quality, with sensitivity thresholds (approx. 0.1 lux) far below the known thresholds for photosynthesis (Bünning and Moser 1969). Therefore, artificial light at night might have been perceived by chryptochromes and disrupted circadian regulation in my experiment. As the net effect of ALAN on the periphyton biomass accrual was negative, these costs apparently outweighed the potential benefits of nocturnal stimulation of photosynthesis.

In **Chapters 3 and 4** I presented evidence that community composition of stream periphyton was altered by LED light at night in communities in the later colonization stages (three to six weeks), even though ALAN did not affect the biomass of periphyton or its autotrophic component (Chapter 2). LED light at night altered concentrations of different pigments in spring and autumn (Chapter 3) and altered taxonomic composition of periphyton in autumn (Chapter 4). These fine-scale impacts were not previously detected in the same communities using *in-situ* fluorometry (the BenthoTorch) (Chapter 2), but required the application of sensitive methods such as high-pressure liquid chromatography (Chapter 3) and DNA metabarcoding (Chapter 4).

Using a pigment analysis, presented in **Chapter 3,** I found that LED light at night decreased the relative abundance of red lineage algae (diatoms and/or chrysophytes) and increased the abundance of cyanobacteria in later developmental stages of stream periphyton in spring. These effects of ALAN were opposite to what I previously found for early developmental stages (Chapter 2), where ALAN increased the proportion of diatoms and decreased the proportion of cyanobacteria. Diatoms are generally considered to be better adapted to grow in low-light environments than cyanobacteria and chrysophytes (Richardson et al. 1983) and thus may be better adapted to use low-level ALAN for photosynthesis. Photosynthetic optima and minimum light requirements for growth differ considerably between individual species (Hill 1996). The opposite trends I found for diatoms and cyanobacteria in the early and the later colonization stages (Chapter 2 and Chapter 3) suggest that the two stages consisted of different taxa with contrasting responses to artificial light at night. The differences in performance at different resource levels between species determine their growth rate under specific conditions and ultimately drive community dynamics (Stevenson 1996). The differences in sensitivity, requirements and performance between the species within the major auto-

trophic groups in periphyton thus seem to play a major role in the community response to ALAN.

In **Chapter 4** I complemented the pigment analysis of stream periphyton communities in later developmental stages with a taxonomic analysis focusing on diatoms and I found that LED light at night altered quantitative community composition (based on relative abundancies) in autumn, but did not affect diversity or qualitative composition, i.e. presence/absence of taxa in the periphyton. Several taxa of diatoms (*Diatoma hyemalis*) and chrysophytes (*Hydrurus foetidus* and a "S*pumella*-like flagellate"), both autotrophic and heterotrophic, were found to taxon-specifically respond to artificial light at night, increasing or decreasing in abundance in autumn. These results provided an insight into responses of the individual taxa and a better understanding how these individual differences shape the response of periphyton to ALAN at the community level. Taxon-specific effects of ALAN emphasized the necessity of taxonomic identification for understanding and predicting potential impacts of ALAN on periphyton.

In my second experimental study, presented in **Chapter 5**, I found that LED light at night (from three to 13 weeks of exposure) decreased the biomass of periphyton in winter in a very different freshwater ecosystem, a lowland agricultural ditch, but high-pressure sodium light at night did not. Here, periphyton was characterized by different taxa (data not presented) and grazing pressure was considered to be low and not an important determinant of periphyton biomass. I argued that the decrease in biomass might be a general response of periphyton to LED light at night across different ecosystems; an effect driven by its spectral composition, in particular the strong emission of blue light by LEDs. Artificial light at night rich in blue wavelengths may interfere with circadian regulation, because blue light is perceived by photoreceptors and mediates entrainment of the circadian clock. High-pressure sodium light, with predominantly yellow wavelengths and typically low blue emission, was found to have no effect on periphyton biomass in winter and summer. I observed a large variation in community composition in periphyton from lit and control sites, but I found no clear patterns that would indicate that artificial light at night by either LED or HPS light significantly affected the community composition of periphyton.

Both of my studies indicated that the ratio of autotrophs to heterotrophs in the periphyton was not altered by ALAN and that altered light regime did not cause photoacclimation in periphyton. Algae can adapt to different light intensities and qualities to maximize photosynthetic efficiency under the available light conditions, by adjusting the cellular content of pho-

tosynthetic pigments and/or changing pigment ratios in the process of photoacclimation (Falkowski and LaRoche 1991). E.g. algae increase the efficiency of light harvesting under limiting daylight conditions by increasing the concentrations of chlorophylls (a response known as "shade adaptation") (Falkowski and LaRoche 1991). Aquatic systems that are exposed to (low intensity) ALAN also experience natural daylight conditions (high intenstiy) and it is not clear whether periphyton would acclimate to ALAN to be able to use it for nocturnal photosynthesis. The ratio of chl *a* to dry mass is also a commonly used indicator for the proportion of autotrophs in the periphyton community (Stevenson 1996). In both of my studies, the ratio of chl *a* to dry mass was not affected by artificial light at night, indicating that neither photoacclimaton nor increase of autotroph proportion were significant in periphyton exposed to ALAN.

6.1.1. The role of season in the effects of ALAN on periphyton

Both experiments showed that season played a significant role in determining periphyton composition, sensitivity and response to ALAN and emphasized the need to consider seasonally-driven variations when assessing and predicting effects of artificial light at night on periphyton. Seasonal variation in light intensity and photoperiod is a strong driver of seasonal succession in periphyton composition, even at constant temperatures (Biggs 1996; Wu 2017) and can reflect in seasonal patterns in photosynthetic capacity (see Laviale et al. 2009). Algal communities in winter and spring are generally considered to be better adapted to low-light environments, compared to communities in summer and autumn (Kirk 1994; Laviale et al. 2009). Periphyton may thus be better adapted to perceive low-level ALAN in winter and spring, both to use low-light for nocturnal photosynthesis and to be more sensitive to disruption of light/dark cycles by nocturnal illumination. In a stream system, LED led to a greater decrease in periphyton biomass in spring (57%, Chapter 2) compared to autumn (43%); in a lowland agricultural ditch there was a strong decrease in biomass in winter (up to 62%, Chapter 5) but the LED experiment was not repeated in another season. Similarly, effects on cyanobacteria were found in spring (a decrease of 57% in early developmental stages and an increase of 17% in later developmental stages) but not in autumn, suggesting that spring taxa were also more sensitive to ALAN. These results seem to support the assumption that seasonal differences in sensitivity to ALAN may be related to the physiological acclimation driven by the light exposure history, suggesting that periphyton may be more sensitive to disturbance by ALAN in winter and spring. Diatoms/chrysophytes were affected both in spring (a decrease of 14% in later developmental stages) and in autumn (diatoms decreased by 11% after

two weeks and increased by 5% after three weeks in early developmental stages and three taxa were found to altered their abundance in later developmental stages), but there were no clear seasonal patterns in impacts of ALAN.

6.1.2. The role of periphyton developmental stage in the effects of ALAN

I found that early developmental stages of periphyton were more sensitive to exposure to artificial light at night, as artificial nocturnal illumination (LED) was found to decrease the biomass only in periphyton in early developmental stages (at two and three weeks of exposure) but not at later developmental stages (three to six weeks) in stream periphyton. In stable flow conditions, periphyton develops into a complex, multi-layered matrix composed of complex forms of large and colonial diatoms and filamentous cyanobacteria. Thick periphyton biofilms quickly attenuate light and are thus resistant to high-light stress (Hill 1996). Laviale et al. (2009) also reported that two week old stream periphyton was more affected by high light stress in the field than four and six week old communities. My results suggest that thicker periphyton biofilms may also be less sensitive to artificial light at night. As periphyton thickness increases with community development over time, the proportion of cells in upper layers that are affected by ALAN to the shaded cells in deeper layers decreases. Thus impacts of ALAN on individual taxa may not be detectable at the community level in later developmental stages. In the second study, a biomass decrease was found under ALAN in winter starting from three weeks of exposure (LED) and remained over 13 weeks of experiment. Exposure of one to four weeks (HPS) in summer did not have an effect on periphyton biomass, but here the type of the light source and high temperatures that stimulated growth in summer were confounding effects in assessing the role of periphyton colonization stage and biofilm thickness in effects of artificial light at night.

6.1.3. Choosing an appropriate method for assessment of effects of ALAN on periphyton

All three techniques I applied in my studies, *in situ* fluorometry (the BenthoTorch), high pressure liquid chromatography and DNA metabarcoding were shown as suitable for assessment of effects of artificial nocturnal illumination on periphyton and the choice of an appropriate method should be based on the specific aims and the desired resolution level in the study. The BenthoTorch is promoted as an inexpensive, rapid method of assessment of biomass and periphyton community composition and in Chapter 2 I showed it can also be used for quick assessment of changes in biomass and community composition induced by

artificial light at night. However, I found that fine-scale alterations in community composition such as pigment or taxonomical composition might be missed with this method. Analysis of pigment composition with high-pressure liquid chromatography (Chapter 3), a widely applied method for assessment of mixed algal assemblages, was found to be more sensitive and suitable for detection of ALAN-induced changes at the community level. While pigment composition analysis may be useful to determine whether periphyton community is significantly affected by artificial nocturnal illumination, a taxonomic identification remains necessary to disentangle potentially confounding effects of intracellular changes in pigment concentrations and relate the trends in pigments to taxonomic categories (Chapter 4). As responses to ALAN can be species-specific (Chapter 4), taxonomic identification is necessary for understanding and predicting the impacts of ALAN, as it provides detailed insight into species composition and community diversity.

Changes in taxonomic composition tend to be the most sensitive response of periphyton to environmental change (Smol and Stoermer 2010). Benthic diatoms rapidly respond to environmental stress by changing metabolic rates, however, if moderate environmental alteration persists for a longer time, communities quickly adapt by changing taxonomic composition observed as decline of sensitive taxa and accumulation of taxa that better tolerate the new conditions (Smol and Stoermer 2010). Thus, altered community composition is likely to be more sensitive indicator of long-term impacts of artificial light at night, rather than a change in biomass or metabolism and this is something to consider when choosing appropriate analytical method.

6.2. Implications for ecosystem functions in illuminated ecosystems

By decreasing periphyton biomass, artificial light at night may reduce oxygen production and food quantity in aquatic food webs and influence nutrient and carbon turnover in aquatic systems; while changes in the proportion of diatoms and cyanobacteria induced by ALAN may alter the food quality of the periphyton and influence energy fluxes to higher trophic levels. Periphyton is an important ecosystem component in clear, shallow waters including streams, ponds, wetlands, shallow coastal waters and lakes. It often dominates primary production and forms the base of the food web in small to mid-sized streams as well as agriculturally influenced streams and ditches (Biggs 1996; Breuer et al. 2016). Positioned at the base of the aquatic food web, as a critical link between abiotic and biotic ecosystem components, periphyton plays a key role in biochemical processes such as carbon and nutrient

cycling and influences energy fluxes toward higher trophic levels (Lowe and Pan 1996). Alterations of periphyton communities induced by artificial light at night may thus profoundly influence critical ecosystem functions and cascade through the ecosystem.

The finding that periphyton in early developmental stages is more sensitive to artificial light at night, as suggested by the decrease in biomass that was observed only for periphyton in early developmental stages, implies that aquatic systems dominated by periphyton in early developmental stages, e.g. those with frequent physical disturbances that reset the successional development of periphyton, may also be more sensitive to ALAN than systems with more stable conditions. Further work is needed to test whether this is true in natural ecosystems and what the implications for the ecosystem processes and ecosystem services are.

ALAN may also alter nutritional composition of periphyton in early and later developmental stages differently, by influencing their composition in contrasting ways. The nutritional quality of algae is determined by their biochemical composition, in particular the content of polyunsaturated fatty acids (PUFA) (see Cashman et al. 2013; Guo et al. 2016). PUFAs are essential nutrients for animals, as they are not able to synthesize them *de novo* but depend on their intake through food. In streams and rivers PUFAs are mostly concentrated in the periphyton. Diatoms are rich in polysaccharides and omega-3 polyunsaturated fatty acids of high nutritional value and are therefore the preferred food source for aquatic macroinvertebrates (Guo et al. 2016; Smol and Stoermer 2010). Larger diatoms have higher nutrient uptake rates (Hill et al. 2011) and competitive advantage for light in the periphyton matrix (Hudon and Bourget 1983); they are more sensitive to grazing (Biggs 1996) and thus convey energy to consumers more effectively than smaller taxa (Irwin et al. 2006). *Hydrurus foetidus*, an abundant taxon in streams worldwide, is also an important food source for aquatic consumers (Nicholls and Wujek 2003). In contrast, cyanobacteria lack many important PUFAs and are thus considered as low-quality food. I found that artificial light at night increased the proportion of diatoms and decreased the proportion of cyanobacteria in periphyton in early developmental stages, while the opposite was observed in later developmental stages, i.e. decrease in diatoms and increase in cyanobacteria, along with the decrease of abundance of the dominant taxon, *H. foetidus*. ALAN may thus increase the nutritional quality in early, but decrease it in later stages of periphyton development, with important implications for secondary production. A higher nutritional quality of algae was shown to support higher secondary production, growth and reproduction of consumers (see Guo et al. 2016). To what extend the ALAN-induced changes in periphyton community composition alter periphyton nutritional quality for

primary consumers and influence energy flow through aquatic food webs, remains to be determined.

6.3. Implications for light pollution policy and regulation

In the different studies I found that light levels that do not contribute to photosynthesis can still affect aquatic primary producers. This adds to the existing literature showing that aquatic ecosystems are sensitive to nocturnal illumination (e.g. Hölker et al. 2015; Perkin et al. 2014b). Aquatic ecosystems are frequently intentionally, e.g. for aesthetic aspects, or unintentionally illuminated. In areas with high ecological values this should be avoided as it is clear that illumination, even at low light levels, impacts the ecosystem. A possible mitigation measure is the use of light sources that have very limited emissions in the blue part of the spectrum such as high pressure sodium light. In my study this did not affect periphyton. Effects of high pressure sodium light on insects are highly discussed and at least some authors suggest HPS affects insects less than LED (e.g. Pawson and Bader 2014), as insects are primarily attracted to short wavelength light (van Langevelde et al. 2011). However for fish the opposite might be true due to higher suppression of melatonin by light with predominantly long wavelengths, i.e. red light than with short wavelengths, e.g. blue light (Brüning et al. 2016).

Many aspects of the impact of artificial light on aquatic ecosystems are still unclear and good ways to mitigate the impact still has to be developed, but there are sufficient indications that artificial light negatively impact aquatic ecosystems. Given that illumination of aquatic ecosystems is not essential or intended in most cases, the installation of artificial lights close to aquatic systems should be avoided whenever possible, as far as the security and safety requirements allow (Schroer and Hölker 2016). Generally, ALAN should only be used with the lowest intensity required for its use and with the least impacting spectral composition. If illumination of certain areas near waterbodies cannot be avoided, it is very feasible to direct light to where it’s needed using modern well-designed luminaires (Schroer and Hölker 2016). This should become common practice in lighting design and might be stimulated by guidelines and policies on light pollution.

6.4. Suggestions for future research

This thesis provides evidence that artificial light at night can significantly decrease the biomass of periphyton and alter the proportions of several autotrophic groups within periphyton communities in a real-world context. The mechanisms behind the observed impacts, however, remain unclear. Several mechanisms how ALAN may impact physiological processes to decrease periphyton biomass were discussed in detail in Chapters 2 and 5 and briefly earlier in this chapter. Whether ALAN indeed disrupts circadian regulation and what processes are affected by the disruption of light/dark cycles or the lack of darkness, remains to be determined. If the light levels applied in our experiment are too low to provide energy for photosynthetic production but high enough to maintain the photosynthetic machinery active at night, it can be expected that a threshold exist, where ALAN will supply enough energy for photosynthetic production and the net effect on community biomass could be reversed. That threshold seems to lie between 20 lux and 71 lux, that was shown to stimulate nocturnal photosynthesis (Hölker et al. 2015). Similarly, a lower threshold is also likely to exist, where light intensity is too low to have any biological effect, and a study by Poulin et al. (2013) suggests this value to be lower than 6.6 lux. Minimum light requirements for photosynthesis are not accurately described in the literature; they substantially differ between species and are considered to be between 0.1 and 50 lux. This is a wide range of light intensities that are commonly found both in directly illuminated urban and suburban environments and their surroundings indirectly illuminated by light scattered as sky glow (Kyba et al. 2011). Furthermore, photosynthetic optima differ between species and thus may vary within mixed algal assemblages such as periphyton and phytoplankton, depending on its species composition (Wellnitz and Rinne 1999). Determining these values for mixed, diverse communities is a challenging task. Narrowing the range in which these thresholds can be expected to lie for different aquatic systems with characteristic communities, would provide a basis for development of clearer predictions regarding impacts and consequences of artificial nocturnal illumination on organisms and ecosystem functions in illuminated waters.

Without a better mechanistic understanding, it is hard to predict the implications and consequences of long-term exposure to artificial light at night and its interaction with other factors, e.g. grazing or other anthropogenic stressors such as eutrophication or climate change. Artificial light at night is often only one of many anthropogenic factors that simultaneously act and in urban waters and may interact to have unanticipated effects on the communities. Urbanization impacts aquatic primary producers in multiple ways: increased light and nutrient input often stimulate algal biomass accrual, while immobility and frequent disturbance of

streambed, metal accumulation in sediments and herbicides in the water can limit biomass accrual (Paul and Meyer 2001 and references therein). Frequent disturbances that reset periphyton succession might keep periphyton in urban waters in early developmental stages that were shown to be sensitive to artificial light at night. Furthermore, a high content of suspended solids and dissolved organic carbon, often associated with urban waters, increases water turbidity thus lowering light penetration to the underwater surfaces (Paul and Meyer 2001), potentially limiting light availability for benthic primary producers, but also limiting their exposure to ALAN. Increased oxygen demand from chemical effluents, typical for urbanized waters, may deplete dissolved oxygen from the water. A decrease of periphyton biomass by artificial light at night or other factors of urbanization may further enhance this effect, contributing to hypoxia and degrading ecosystem functions in urban streams.

Not only autotrophic, but also heterotrophic components of periphyton can be affected by artificial light at night. Autotrophs and heterotrophs in periphyton are closely associated and interact and develop interdependently (Barranguet et al. 2005). Periphyton research often focuses on one of these components, while their interactions are rarely considered (Barranguet et al. 2005). Heterotrophic bacteria in periphyton feed on algal exudates and rely on algae for habitat structure. An increase in light to nutrient ratio is predicted to increase bacterial growth rates and the ratio of bacterial to algal biomass (Hill et al. 2011 and references therein). In this thesis I mostly focused on the autotroph component of the periphyton; however I found that the relative abundance of a heterotrophic chrysophyte from the *Spumella*-like flagellates group increased under artificial light at night (Chapter 4). Hölker et al. (2015) also reported that ALAN increased or decreased the relative abundance of several heterotrophic taxa in microbial communities from sediments. My results confirm that artificial light at night impacts heterotrophs also in the periphyton and emphasize the need for better understanding of underlaying mechanisms of ALAN impacts on heterotrophs and the consequences for periphyton communities.

6.5. Conclusions

In this thesis I found that artificial nocturnal illumination, in particular LED light at night, can have profound and complex impacts on communities of benthic primary producers, the periphyton, by decreasing their biomass and altering abundance of several autotrophic groups in periphyton in a real-world context. Ecological effects of artificial light at night on aquatic primary producers have rarely been considered in previous research. Potential impacts

on communities of aquatic primary producers and implications for ecosystem functioning have been underestimated, as often only effects on photosynthesis are discussed. It is likely that other more sensitive processes than photosynthesis, such as the biological clock, are impacted by nocturnal artificial illumination and responsible for the effects I observed. As periphyton is a fundamental component of many aquatic ecosystems, decreased biomass and altered community composition in illuminated waters can potentially affect critical ecosystem functions. Light intensity, spectral composition, differences between the ecosystems and seasonal variations all play a role in impacts of artificial nocturnal illumination in a way that is not yet clear. This urges for better understanding of underlying mechanisms of impacts of artificial light at night on aquatic primary producers and of how these impacts translate to ecosystem functions, in order to identify and mitigate adverse ecological effects of light pollution.

References (General discussion)

Altermatt, F., and D. Ebert. 2016. Reduced flight-to-light behaviour of moth populations exposed to long-term urban light pollution. Biol. Lett. **12:** 20160111.

Barranguet, C., B. Veuger, S. A. M. Van Beusekom, P. Marvan, J. J. Sinke, and W. Admiraal. 2005. Divergent composition of algal-bacterial biofilms developing under various external factors. Eur. J. Phycol. **40:** 1-8.

Becker, A., A. K. Whitfield, P. D. Cowley, J. Jarnegren, and T. F. Naesje. 2013. Potential effects of artificial light associated with anthropogenic infrastructure on the abundance and foraging behaviour of estuary-associated fishes. J. Appl. Ecol. **50:** 43-50.

Biggs, B. J. F. 1996. Patterns in benthic algae of streams, p. 31-56. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Breuer, F., P. Janz, E. Farrelly, and K.-P. Ebke. 2016. Environmental and structural factors influencing algal communities in small streams and ditches in central Germany. J. Freshwat. Ecol.**:** 1-19.

Brüning, A., F. Hölker, S. Franke, W. Kleiner, and W. Kloas. 2016. Impact of different colours of artificial light at night on melatonin rhythm and gene expression of gonadotropins in European perch. Sci. Total Environ. **543:** 214-111.

Brüning, A., F. Hölker, S. Franke, T. Preuer, and W. Kloas. 2015. Spotlight on fish: Light pollution affects circadian rhythms of European perch but does not cause stress. Sci. Total Environ. **511:** 516-522.

Bünning, E., and I. Moser. 1969. Interference of moonlight with the photoperiodic measurement of time by plants, and their adaptive reaction. Proc. Natl. Acad. Sci. USA **62:** 1018-1022.

Cashman, M. J., J. D. Wehr, and K. Truhn. 2013. Elevated light and nutrients alter the nutritional quality of stream periphyton. Freshwat. Biol. **58:** 1447–1457.

Davies, T. W., M. Coleman, K. M. Griffith, and S. R. Jenkins. 2015. Night-time lighting alters the composition of marine epifaunal communities. Biol. Lett. **11:** 20150080-20150080.

Falkowski, P. G., and J. LaRoche. 1991. Acclimation to spectral irradiance in algae. J. Phycol. **27:** 8-14.

Gaston, K. J., J. Bennie, T. W. Davies, and J. Hopkins. 2013. The ecological impacts of nighttime light pollution: A mechanistic appraisal. Biol. Rev. **88:** 912-927.

Gaston, K. J., M. E. Visser, and F. Hölker. 2015. The biological impacts of artificial light at night: The research challenge. Phil. Trans. Roy. Sci. B. **370:** 20140133.

Guo, F., M. J. Kainz, F. Sheldon, and S. E. Bunn. 2016. The importance of high-quality algal food sources in stream food webs – current status and future perspectives. Freshwat. Biol. **61:** 815–831.

Hegemann, P., M. Fuhrmann, and S. Kateriya. 2001. Algal sensory photoreceptors. J. Phycol. **37:** 668–676.

Hill, W. R. 1996. Effects of light, p. 121-149. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Hill, W. R., B. J. Roberts, S. N. Francoeur, and S. E. Fanta. 2011. Resource synergy in stream periphyton communities. J. Ecol. **99:** 454–463.

Hölker, F., C. Wurzbacher, C. Weissenborn, M. T. Monaghan, S. I. J. Holzhauer, and K. Premke. 2015. Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. Phil. Trans. Roy. Sci. B. **370:** 20140130.

Hudon, C., and E. Bourget. 1983. The effect of light on the vertical structure of epibenthic diatom communities. Bot. Mar. **26:** 317-330.

Irwin, A. J., Z. V. Finkel, O. M. E. Schofield, and P. G. Falkowski. 2006. Scaling-up from nutrient physiology to the size-structure of phytoplankton communities. Journal of Phytoplankton Research **28:** 459-471.

Kirk, J. T. O. 1994. Light and photosynthesis in aquatic ecosystems. Cambridge University Press.

Kyba, C. C. M., T. Ruhtz, J. Fischer, and F. Hölker. 2011. Cloud coverage acts as an amplifier for ecological light pollution in urban ecosystems. Plos One **6:** e17307.

Laviale, M., J. Prygiel, Y. Lemoine, A. Courseaux, and A. Creach. 2009. Stream periphyton photoacclimation response in field conditions: Effect of community development and seasonal changes. J. Phycol. **45:** 1072-1082.

Lowe, R. L., and Y. Pan. 1996. Benthic algal communities as biological monitors, p. 705-739. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Meyer, L. A., and S. M. P. Sullivan. 2013. Bright lights, big city: Influences of ecological light pollution on reciprocal stream-riparian invertebrate fluxes. Ecol. Appl. **23:** 1322-1330.

Nicholls, K. H., and D. E. Wujek. 2003. Chrysophycean Algae. *In* J. D. Wehr and R. G. Sheath [eds.], Freshwater algae of North America: Ecology and classification. Academic Press.

Paul, M. J., and J. L. Meyer. 2001. Streams in the urban landscape. Annu. Rev. Ecol. Syst. **32:** 333-365.

Pawson, S. M., and M. K. F. Bader. 2014. LED lighting increases the ecological impact of light pollution irrespective of color temperature. Ecol. Appl. **24:** 1561-1568.

Perkin, E. K., F. Hölker, J. S. Richardson, J. P. Sadler, C. Wolter, and K. Tockner. 2011. The influence of artificial light on stream and riparian ecosystems: Questions, challenges, and perspectives. Ecosphere **2:** 1-16.

Perkin, E. K., F. Hölker, and K. Tockner. 2014a. The effects of artificial lighting on adult aquatic and terrestrial insects. Freshwat. Biol. **59:** 368-377.

Perkin, E. K., F. Hölker, K. Tockner, and J. S. Richardson. 2014b. Artificial light as a disturbance to light-naive streams. Freshwat. Biol. **59:** 2235-2244.

Poulin, C., F. Bruyant, M.-H. Laprise, A. M. Cockshutt, J. M.-R. Vandenhecke, and Y. Huot. 2014. The impact of light pollution on diel changes in the photophysiology of *Microcystis aeruginosa*. J. Plankton Res. **36:** 286-291.

Raven, J. A., and C. S. Cockell. 2006. Influence on photosynthesis of starlight, moonlight, planetlight, and light pollution (reflections on photosynthetically active radiation in the universe). Astrobiology **6:** 668-675.

Richardson, K., J. Beardall, and J. A. Raven. 1983. Adaptation of unicellular algae to irradiance: An analysis of strategies. New Phytol. **93:** 157-191.

Riley, W. D., B. Bendall, M. J. Ives, N. J. Edmonds, and D. L. Maxwell. 2012. Street lighting disrupts the diel migratory pattern of wild Atlantic salmon, *Salmo salar* L., smolts leaving their natal stream. Aquaculture **330:** 74-81.

Schroer, S., and F. Hölker. 2016. Impact of lighting on flora and fauna, p. 1-33. *In* R. Karlicek, C.-C. Sun, G. Zissis and R. Ma [eds.], Handbook of advanced lighting technology. Springer International Publishing.

Smol, J. P., and E. F. Stoermer. 2010. The Diatoms: Applications for the environmental and earth sciences. Cambridge University Press.

Stevenson, R. J. 1996. An introduction to algal ecology in freshwater benthic habitats. *In* R. J. Stevenson, M. L. Bothwell and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

van Langevelde, F., J. A. Ettema, M. Donners, M. F. WallisDeVries, and D. Groenendijk. 2011. Effect of spectral composition of artificial light on the attraction of moths. Biol. Conserv. **144:** 2274–2281.

Wellnitz, T., and B. Rinne. 1999. Photosynthetic response of stream periphyton to fluctuating light regimes. J. Phycol. **35:** 667-672.

Wu, Y. 2017. Periphyton: Functions and application in environmental remediation. Elsevier.

Appendix A: Supplementary material for Chapter 2

Figure S1. Estimators of biomass measured with the BenthoTorch (BT) compared to pigment concentrations detected by high-performance liquid chromatography (HPLC) and the values obtained by dry mass (DM). Data on Log scale, except for plot d.

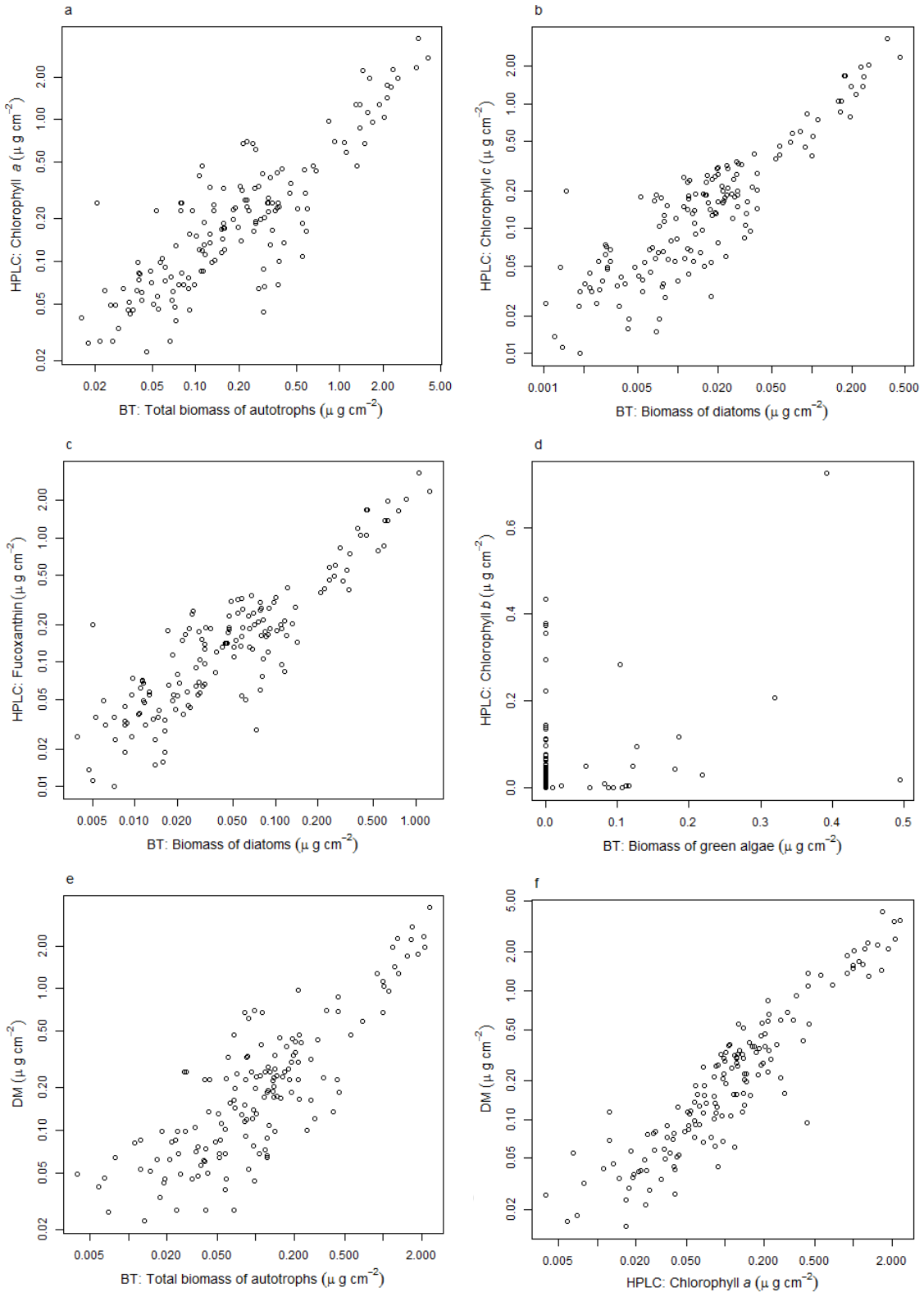

Table S1. Environmental parameters measured in the flumes in the two seasons (continues on next page).

Spring	31.03. (before)					07.04. (one week)				
Flume	A	B	C	D	E	A	B	C	D	E
Conductivity (µS cm^{-1})	108.8	108.9	108.9	109.4	109.7	90.9	90.9	91.0	91.4	91.2
Temperature (°C)	7.3	7.2	7.2	7.1	7.1	6.3	6.3	6.3	6.3	6.3
Oxygen (mg L^{-1})	11.87	11.82	11.89	11.87	11.95	11.85	11.76	11.71	11.76	11.71
Oxygen (%)	105.0	104.5	105.1	104.7	105.4	102.1	101.8	100.9	101.4	101.1
pH	7.7	7.8	7.8	7.8	7.8	7.5	7.6	7.6	8.3	5.3
Turbidity (NTU)	1.16	1.27	1.25	1.46	1.35	1.52	1.38	1.56	1.72	1.56
Velocity (m s^{-1})	NA	NA	NA	NA	NA	0.3	0.2	0.3	0.2	0.3
Date	14.04. (two weeks)					23.04. (after/three weeks)				
Flume	A	B	C	D	E	A	B	C	D	E
Conductivity (µS cm^{-1})	79.7	80.1	80.0	79.6	79.8	82.7	82.7	82.7	82.7	82.7
Temperature (°C)	5.9	5.9	5.9	5.9	5.9	6.8	6.8	6.8	6.8	6.8
Oxygen (mg L^{-1})	11.71	11.56	11.66	11.58	11.33	10.53	10.10	10.13	10.01	9.86
Oxygen (%)	100.3	98.9	99.9	99.0	97.0	93.9	92.2	90.0	88.6	86.6
pH	7.8	7.8	7.8	7.8	7.8	8.0	7.8	7.9	7.9	8.2
Turbidity (NTU)	1.94	1.92	1.83	1.70	1.75	1.83	1.81	1.97	1.84	1.93
Velocity (m s^{-1})	0.5	0.4	0.3	0.3	0.4	0.5	0.4	0.3	0.3	0.3

Autumn	24.09. (before)					01.10. (one week)				
Flume	A	B	C	D	E	A	B	C	D	E
Conductivity (µS cm^{-1})	143.6	143.2	143.1	143.1	143.1	147.6	147.7	147.7	147.6	147.6
Temperature (°C)	10.7	10.65	10.7	10.7	12.05	12.8	12.8	12.8	12.8	12.8
Oxygen (mg L^{-1})	9.18	9.23	9.36	9.31	9.72	10.10	10.11	10.55	10.68	10.43
Oxygen (%)	88.1	89.2	90.3	89.0	92.4	101.6	101.3	105.7	106.9	104.6
pH	8.0	8.0	8.0	8.0	8.0	8.1	8.0	8.2	8.1	8.1
Turbidity (NTU)	0.37	0.53	0.47	0.36	0.25	NA	NA	NA	NA	NA
Velocity (m s^{-1})	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3
Date	08.10. (two weeks)					16.10. (after/three weeks)				
Flume	A	B	C	D	E	A	B	C	D	E
Conductivity (µS cm^{-1})	149.1	149.0	149.0	149.1	149.1	149.8	149.5	149.9	150.2	150.0
Temperature (°C)	11.9	11.9	11.9	11.9	11.9	11.7	11.7	11.7	11.7	11.7
Oxygen (mg L^{-1})	10.59	10.61	10.63	10.56	10.29	10.25	10.27	10.23	10.33	10.29
Oxygen (%)	105.0	104.7	105.1	103.5	101.9	102.0	102.2	102.0	103.6	102.4
pH	8.1	8.0	8.1	8.0	8.1	7.7	7.7	7.7	7.7	7.7
Turbidity (NTU)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Velocity (m s^{-1})	0.4	0.4	0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.4

Table S2. Absolute biomass of major autotrophic groups (µg cm^{-2}) measured with the BenthoTorch in two experimental seasons in control (D) and lit (L) developing periphyton (single measurements n = 1265) and pre-established periphyton (single measurements n = 1263). Median and 95% confidence intervals.

	Two weeks		Three weeks	
	D	L	D	L
Developing periphyton				
Spring				
Diatoms	0.06 (0 - 0.95)	0.11 (0 - 0.82)	0.81 (0.02 - 10.26)	0.32 (0.03 - 3.62)
Green algae	0.02 (0 - 0.62)	0.03 (0 - 0.31)	0 (0 - 2.29)	0.04 (0 - 4.66)
Cyanobacteria	0 (0 - 0.14)	0 (0 - 0.12)	0.11 (0 - 0.74)	0.02 (0 - 0.41)
Autumn				
Diatoms	0.05 (0 - 0.29)	0.03 (0 - 0.72)	0.07 (0.01 - 0.74)	0.08 (0.01 - 0.88)
Green algae	0.01 (0 - 0.11)	0.01 (0 - 0.07)	0 (0 - 0.12)	0 (0 - 0.17)
Cyanobacteria	0 (0 - 0.04)	0 (0 - 0.19)	0 (0 - 0.23)	0 (0 - 0.12)
Pre-established periphyton				
	Before		After	
	D	L	D	L
Spring				
Diatoms	0.08 (0 - 0.48)	0.09 (0.01 - 0.56)	0.33 (0.04 - 5.02)	0.32 (0.05 - 5.77)
Green algae	0.06 (0 - 0.68)	0.05 (0 - 0.06)	0 (0 - 0.93)	0 (0 - 1.67)
Cyanobacteria	0 (0 - 0.03)	0 (0 - 0.04)	0.05 (0-0.82)	0.03 (0 - 0.80)
Autumn				
Diatoms	0.03 (0 - 0.16)	0.05 (0 - 0.37)	0.16 (0.03 - 0.53)	0.19 (0.02 - 0.70)
Green algae	0.01 (0 - 0.05)	0.01 (0 - 0.09)	0 (0 - 0.09)	0 (0 - 0.98)
Cyanobacteria	0 (0 - 0.03)	0 (0 - 0.02)	0.03 (0 - 0.32)	0.04 (0 - 0.32)

Appendix B: Supplementary material for Chapter 4

Figure S2. Krona charts generated from read abundance matrix averaged for treatment x time groups (n = 3) for visualization of taxa identified by DNA metabarcoding in the periphyton communities in spring. Data based on taxonomical assignment using OBI tools pipeline with maximum depth of 11.

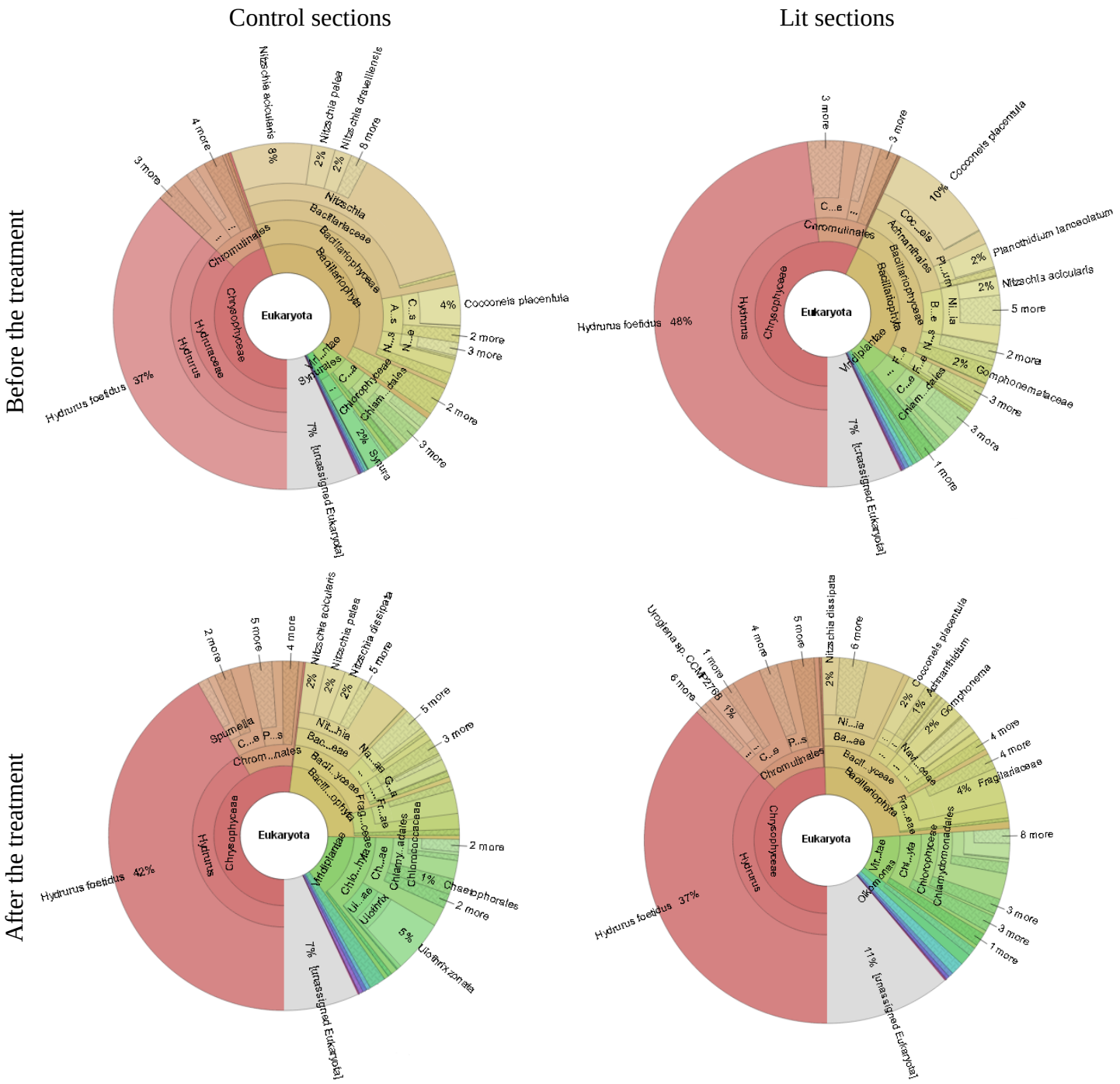

Figure S3. Krona charts generated from read abundance matrix averaged for treatment x time groups (n = 3) for visualization of taxa identified by DNA metabarcoding in the periphyton communities in autumn. Data based on taxonomical assignment using OBI tools pipeline with maximum depth of 11.

Control sections

Lit sections

Before the treatment

After the treatment

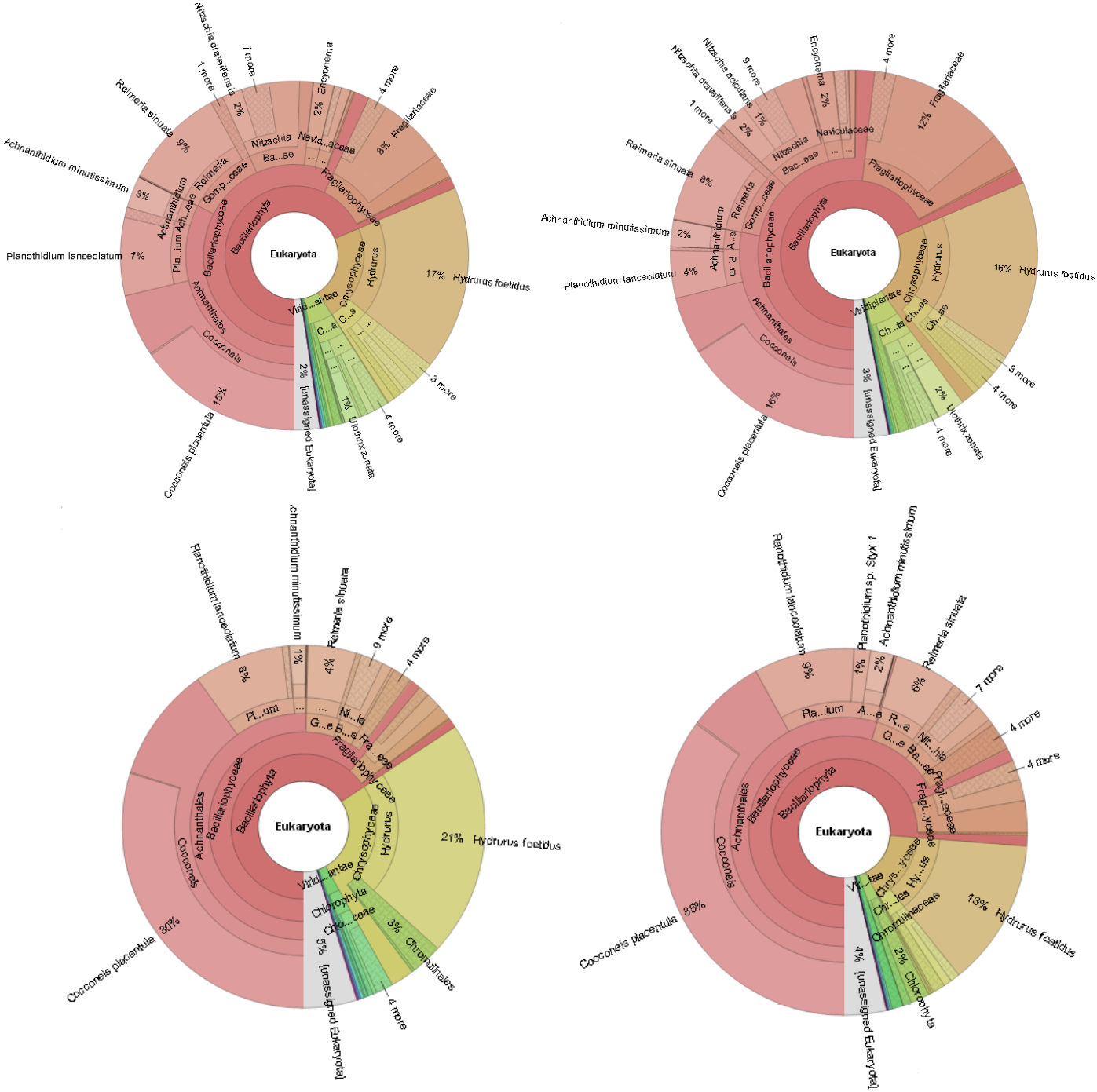

Table S3. List of taxa identified by DNA metabarcoding (OTU assignment using UPARSE and BLAST) that significantly differed in abundance between spring and autumn periphyton (permutation analysis based on the full read abundance matrix randomly subsampled 1000 times and Kruskal-Wallis tests performed for each taxonomic group. All $Pq < 0.05$). Number of reads averaged per season ($n = 48$).

	Spring		**Autumn**	
OTUs (taxa)	No. of reads	% of total reads	No. of reads	% of total reads
Chrysophytes				
Hydrurus foetidus	27917	94.80	10043	50.09
Diatoms				
Achnanthidium minutissimum	3	0.01	83	0.41
Cocconeis placentula	68	0.23	5836	19,8
Cocconeis pediculus	1	0.003	33	0.16
Diatoma hyemalis	13	0.04	127	0.63
Encyonema sp.	1	0.003	38	0.19
Planothidium lanceolatum	7	0.02	622	3.1
Reimeria sinuata	2	0.01	784	3.91
Ulnaria acus	1	0.003	83	0.41

Appendix C: Supplementary material for Chapter 5

Figure S4. Correlation biplots of PCA (principal component analysis) based on relative pigment composition of phytoplankton founder communities, normalized to chlorophyll *a*, originating from the two control sites (Control 1 and Control 2) and the Lit site, under high-pressure sodium lamps (HPS) (a, b) or LED lamps (c) in summer (a) and in winter (b, c) (n = 12). Planes of the first two PC axes explain 69% (a), 78% (b) and 93% (c) of variation in the data. The pigments included in the analysis are: chlorophyll *b* (chl b), chlorophyll *c* (chl c), fucoxantin (fucox), violaxanthin (violax), diadinoxanthin (diadinox), neoxanthin (neox), zeaxanthin (zeax), alloxanthin (allox) and lutein. Arrows show correlation between original pigment variables with PC axes and all indicate significant relationship ($p < 0.0001$).

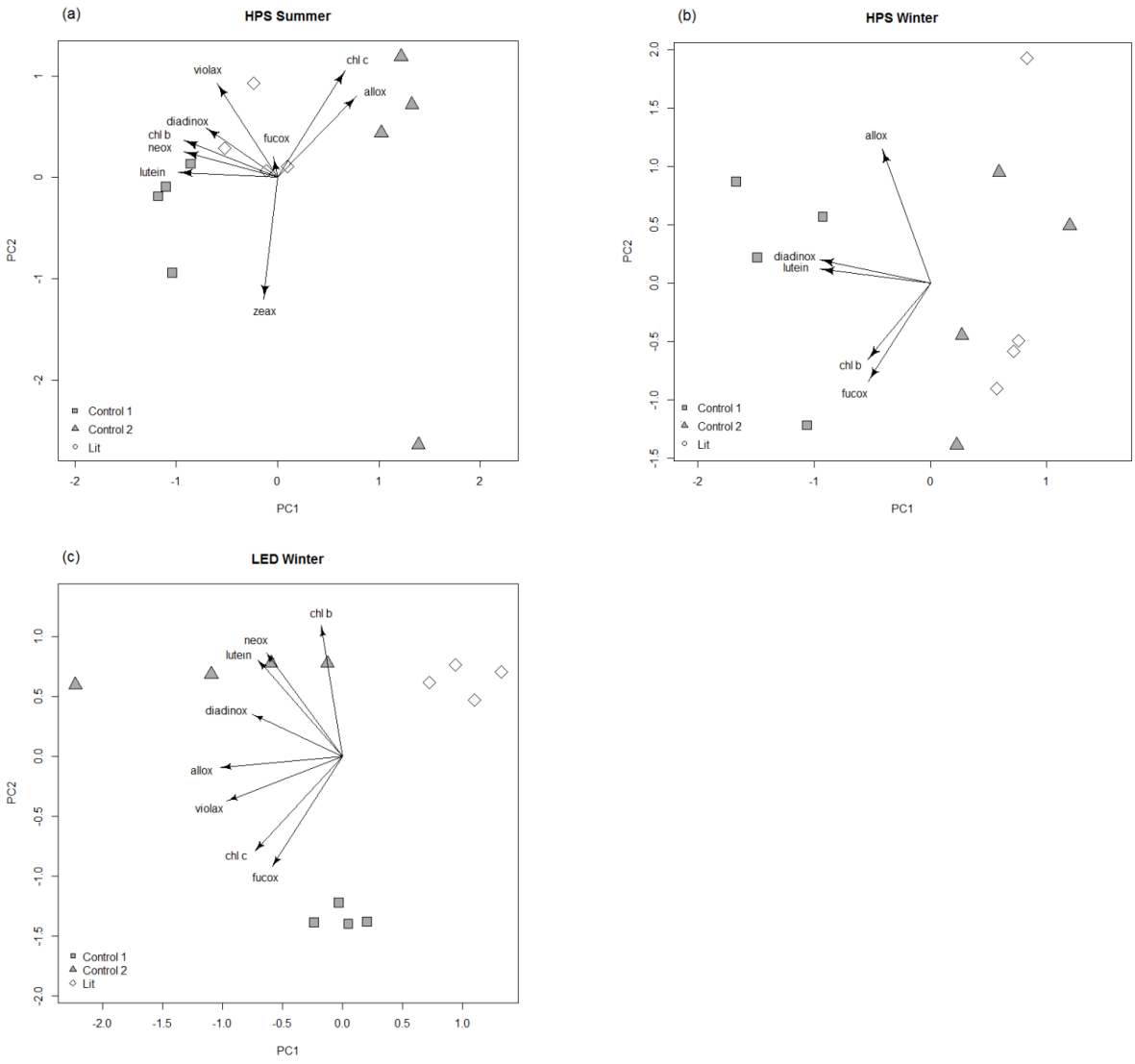

Figure S5. Factor scores of the first axes of the principal component analysis (PCA) for the relative pigment concentrations of phytoplankton, normalized to chlorophyll *a*, originating from the two control sites and the Lit site developed under high-pressure sodium lamps (HPS; a, b) or LED lamps (c) in summer (a) and in winter (b, c) (*n* = 12).

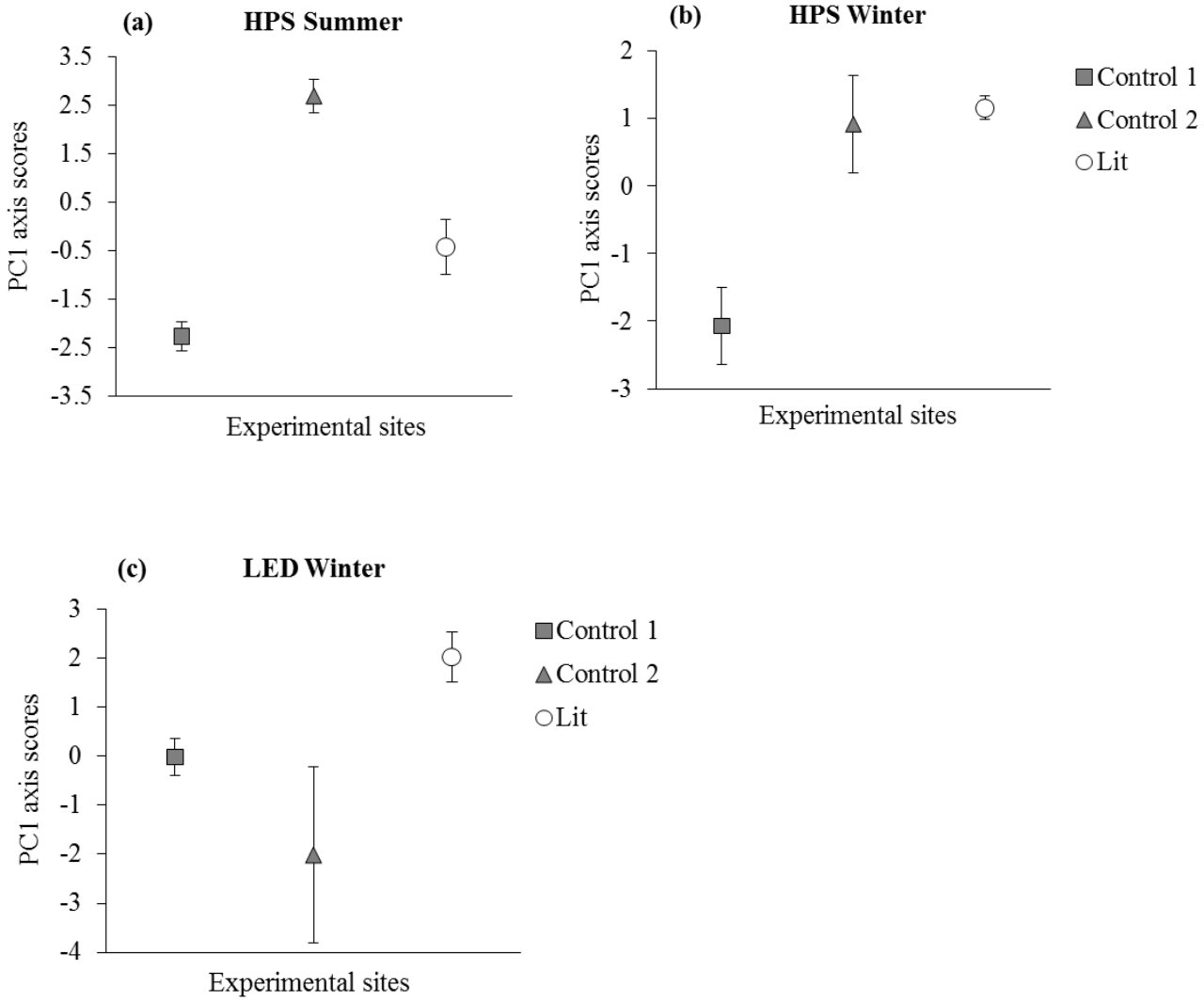

Figure S6. Factor scores of the first axes of the principal component analysis (PCA), for the relative pigment concentrations of periphyton, normalized to chlorophyll a, developed at the two control sites and the lit site developed under high-pressure sodium lamps (HPS; a, b) or LED lamps (c) in summer (a, n = 48) and in winter (b, n = 60; c, n = 84).

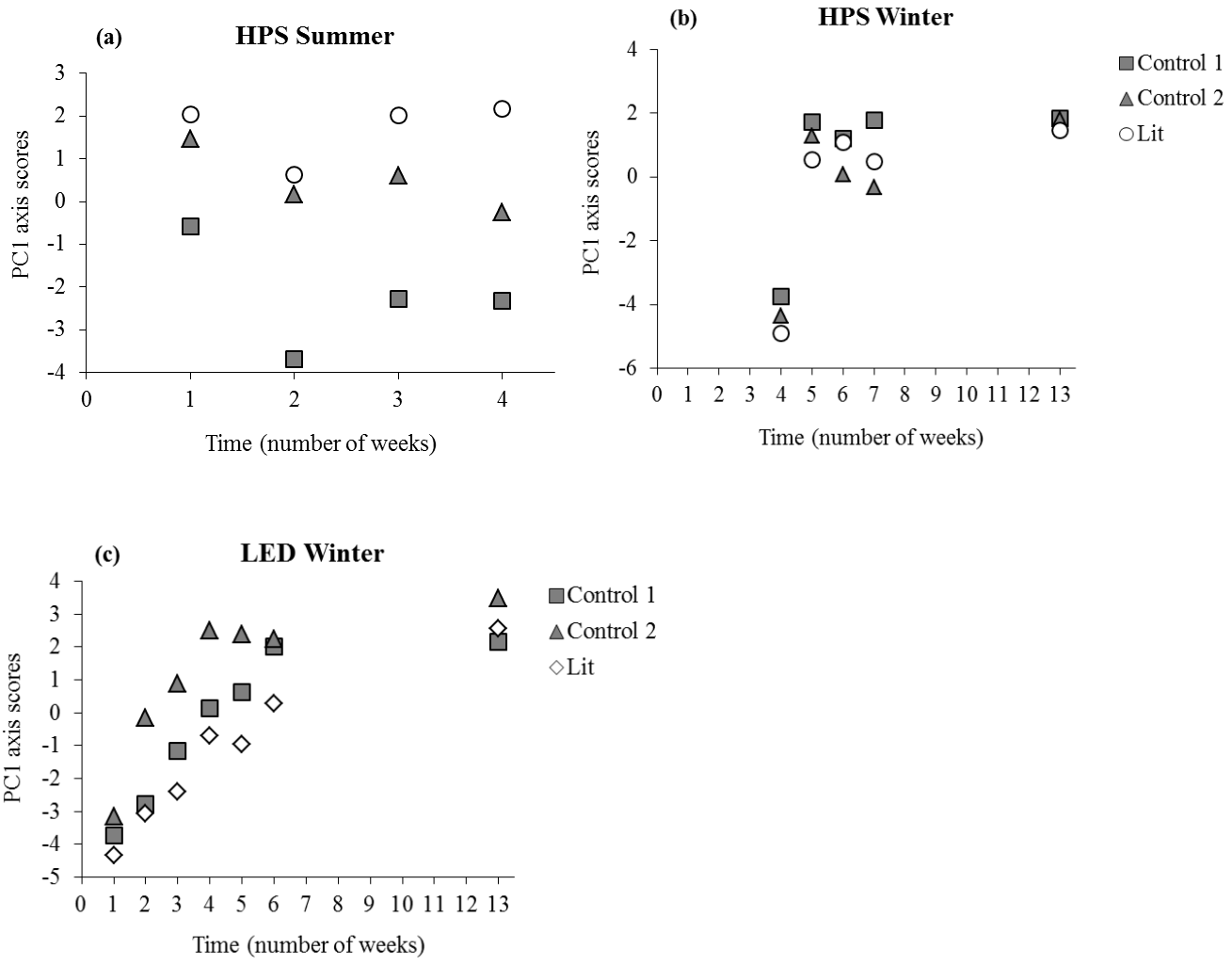

Table S4. Environmental parameters measured with the multi probes at the three experimental sites for the summer sampling under high-pressure sodium lamps (HPS).

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 S5. 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 S6. Environmental parameters measured with the probes at the three experimental sites for the winter sampling under 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 S7. Chemical parameters (µg L^{-1}) measured at the three experimental sites.

	HPS Summer			**HPS Winter**			**LED Winter**		
	Lit	Control 1	Control 2	Lit	Control 1	Control 2	Lit	Control 1	Control 2
Dissolved organic carbon	9.92	11.98	NA	10.33	10.05	NA	17.38	14.88	18.03
Dissolved nitrogen	0.61	0.76	NA	0.77	0.96	NA	1.33	1.16	1.39
Dissolved organic nitrogen	0.59	0.69	NA	0.59	0.64	NA	1.20	0.98	1.24
Nitrate-nitrogen	0.01	0.01	NA	0.01	0.01	NA	0.11	0.04	0.10
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	0.15	0.06
Solubile reactive phosphorus	19.67	35.17	NA	62.00	72.00	NA	15.08	35.94	8.00
Total phosphorus	64.00	90.42	NA	107.75	119.75	NA	63.69	74.33	53.17
Chloride	27.73	16.85	NA	17.75	18.95	NA	20.09	22.83	20.00
Sulphate	86.33	76.25	NA	75.75	67.50	NA	92.33	94.83	96.00
Dissolved silica	3.82	5.76	NA	NA	NA	NA	5.01	5.21	4.92

Table S8. Pigment composition of phytoplankton founder community (µg L^{-1}) originating from the three experimental sites in summer and winter.

	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 S9. 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) (n.d. = not detected).

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
Zeaxanthin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table S10. 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) (n.d. = not detected).

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
Zeaxanthin	0.04	n.d.	n.d.	n.d.	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	0.03	n.d.	n.d.	0.01	0.09

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) (n.d. = not detected).

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
Zeaxanthin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Statement of academic integrity

I hereby certify that the submitted thesis “Effects of artificial light at night on benthic primary producers in freshwaters” is my own work and that all published or other sources of material consulted in its preparation have been indicated. I have clearly pointed out any collaboration that has taken place with other researchers and stated my own personal share in the investigations in the Thesis Outline. I confirm that this work has not been submitted to any other university or examining body for a comparable academic award.

Berlin, 30.03.2017

Maja Grubisic

Acknowledgements

Coming to the end of this exciting journey, I would like to look back and sincerely thank to all the people who have helped me come to where I stand today; who supported me, challenged me and inspired me over the previous years.

I wish to thank the academic board of SMART EMJDP for accepting me into this program. It has been a rewarding experience that enriched me and changed me forever.

I thank my supervisors, Franz Hölker and Michael Monaghan, for agreeing to supervise my project and providing me with necessary support and freedom to learn and grow. A special thank to Franz for introducing me to the COST LoNNe network and making me a part of the global light pollution research community from the beginning on, and to Michael for advice and encouraging words. I thank Cristina Bruno for inspiring energy and taking care of my well-being by taking me along to beautiful hikes that made my time in Trento so enjoyable.

I thank all my collaborators for being a huge support through different phases of this project, especially Stefan Heller for driving and helping me in the field, Gabriel Singer for advising me on statistics and R, Jonas Zimmermann for a kind support and guidance through the DNA laboratory work, Roy van Grunsven for reading and discussing my work over and over and Sibylle Schroer for translating the Zusammenfassung. I thank ex- and current members of Mike's Monday group for helpful discussions and cakes, Thomas Mehner for training my writing skills and Kirsten Pohlmann for running valuable workshops within the IGB doctoral program.

I wish to thank Prof. Dr. Klement Tockner for agreeing to review this thesis.

A lot of love for the SMART family that made me feel like home whether in Berlin, Trento or in London. Thank you guys for all the laughs and emotions! To the 3rd cohort, especially Alessandro Manfrin for the countless hard-working and hard-partying memories we shared over the years and across the globe, Abel Machado for inspiring conversations, actions and bubbly drinks and Navid Marofi for hikes, table-tennis sessions and friendship. Thank you Oleksandra Shumilova for sunshine and colors you were bringing into our office. To all my friends at IGB and beyond that made my days brighter and nights longer, especially Barbara Behounek, Karin Mainikmann, Clara Romero, Roshni Arora, Tarun Bisht, Jaime Gaona, Romain Clement, Demetrio Mora and Nemanja Savic.

I wish to thank Zorka Dulic for being a mentor, a field assistant, a moral support and a friend, who shared with me her knowledge and passion for our little friends behind the microscope and much more. I thank prof. Ruza Mrgic for giving me the ground to develop a passion for biology.

I wouldn't be the same person if it wasn't for my Superheroes: Dusica Radojkovic, Natasa Djurdjevac, Sonja Radak and Olja Milicevic. Thank you girls for the long-lasting friendships.

No words can express the deepest gratitude to my dear parents, Rade and Fina and my brother Igor. Thank you for always believing in me and firmly holding the ladder.

And to many, many others who have touched me and made me who I am today.