Effects of artificial light at night (ALAN) on interactions between aquatic and terrestrial ecosystems

Alessandro Manfrin

Freie Universität Berlin

Berlin, 2017

Effects of artificial light at night (ALAN) on interactions between aquatic and terrestrial ecosystems

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

ALESSANDRO MANFRIN

from Rome, Italy

Berlin, 2017

This thesis work was conducted during the period 29th September 2013 – 21st February 2017, under the supervision of PD. Dr. Franz Hölker (Leibniz-Institute of Freshwater Ecology and Inland Fisheries Berlin), Dr. Michael T. Monaghan (Leibniz-Institute of Freshwater Ecology and Inland Fisheries Berlin), Prof. Dr. Klement Tockner (Freie Universität Berlin and Leibniz-Institute of Freshwater Ecology and Inland Fisheries Berlin), Dr. Cristina Bruno (Edmund Mach Foundation San Michele all´Adige) and Prof. Dr. Geraldene Wharton (Queen Mary University of London). This thesis work was conducted at Freie Universität Berlin, Queen Mary University of London and University of Trento. Partner institutes were Leibniz-Institute of Freshwater Ecology and Inland Fisheries of Berlin and Edmund Mach Foundation of San Michele all´Adige.

1st Reviewer: PD. Dr. Franz Hölker

2nd Reviewer: Prof. Dr. Klement Tockner

Date of defence: 22nd May 2017

Erasmus Mundus
Joint Doctorate Programme
SMART - Science for MAnagement of Rivers and their Tidal systems

The SMART Joint Doctorate Programme

Research for this thesis was conducted with the support of the Erasmus Mundus Programme[1], 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.

---

[1] 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

Acknowledgements

First I want to thank The SMART supervisory board: Prof. Klement Tockner, Prof. Angela Gurnell and Prof. Guido Zolezzi, for giving me the unique opportunity to take part in the SMART doctoral program.

I do not have words to thank my supervisors Dr. Michael T. Monaghan, PD. Dr. Franz Hölker, Prof. Klement Tockner, Dr. Cristina Bruno and Prof. Geraldene Wharton for being a source of inspiration during these three years.

Very special thanks go to the three best colleagues and friends I have ever worked with: Dr. Stefano Larsen, Dr. Roy van Grunsven and (soon Dr.) Maja Grubišić.

I am grateful to the colleagues, students and trainees at the IGB who helped me in the field, in the laboratory, or with fruitful discussions. Particularly I want to thank: Ann-Christin Honnen, Valentyna Inshyna, Viktor Baranov, Babette Pohlmann, Nina-Sophie Weiss, Nadine Weiss, Liliana Lehmann, and many others from the Monday journal/discussion group. Special thanks go to Thomas Mehner, Kate Laskowski, Gabriel Singer, Kirsten Pohlmann and Francesca Pilotto for their help with statistics and for their, necessarily critical, discussions on my research. Special thanks go to Elizabeth Perkin and Abel Machado for their important comments on the manuscripts and to Lisa Angermann for helping me with the german translation of the summary.

I also want to thank Stefan Heller, Lorenzo Forti and Martino Salvaro for their help in setting up of the experiments; and Sibylle Schroer, Anika Brüning, Tobias Degen, Stephanie Holzhauer and the entire “Verlust der Nacht” group for such incredible adventure.

I want to say thank to Stefanie Burkert, Frau Katrin Lhemann, Frau Marlis Lange and Marina Rogato for their precious support on administrative issues.

The colleagues and the other PhD students from IGB and the SMART program. Especially I want to thank Çağrı Gökdemir, Alex Lumsdon, Pascal Bodmer and Oleksandra Shumilova with whom I particularly shared this unique experience.

I want to thank my beloved friends Massimo, Simone, Valeria, Alessio, Ruby, Manolo, Fabrizio, Tommy, Massi, Rene, Fanny, Patty, Carolina, Arianna, Annika, Loredana, Cristina and Benito for their continuous support.

Finally, and most of all, I want to thank my family Fiorella, Emilio and Millie for constantly encouraging me and being always present when I needed.

“Research will work for you, but finding
cheap flights is still an important skill in science, too!”

M. T. M.

Table of contents

Summary

It has become clear that artificial light at night (ALAN) is one of the most widespread human-induced alteration of the landscape. Among consequences of ALAN are alterations of animal behaviour and movement. This can lead to changes in spatial and temporal patterns in species distribution, potentially altering predator-prey relationships within and between ecosystems. Such effects are expected to be substantial near water bodies, where human populations are concentrated. Aquatic systems are connected with their adjacent terrestrial areas via fluxes of nutrients, material and energy in the form of (organic) matter and organisms forming subsidies for the recipient ecosystem. Recent work has demonstrated how anthropogenic alterations of aquatic systems can “resonate” into the adjacent terrestrial zones via altered prey subsidy quality and quantity. However, the extent to which freshwater-to-terrestrial subsidy fluxes are affected by ALAN is currently unknown.

In this thesis, I conducted three field studies in two different ecosystems. In a first study, conducted in artificial flumes of a sub-alpine stream, I investigated the effect of ALAN on riverine aquatic macroinvertebrate communities. In a second study, conducted in an agricultural drainage ditch system, I investigated whether the effect of ALAN can propagate from the aquatic to the terrestrial ecosystem via altered aquatic insect subsidies to riparian invertebrate predators and scavengers. In a third study, conducted in the same ditch experimental field, I analysed the effect of these altered subsidies on the diet of the riparian invertebrate predators and scavengers.

The first study showed that exposure to ALAN for one week affected abundance and taxonomical and functional composition of benthic invertebrate communities in the stream-side flumes. Chironomidae and *Baetis* spp. were 4 times more abundant after one week under ALAN than in natural dark conditions. Analysing functional feeding traits, scrapers were 1.5 times more abundant under ALAN than in natural dark conditions while filterers were half as abundant when exposed to ALAN. These results suggest that ALAN inhibited drifting behaviour of Chironomidae, *Baetis* spp. and scrapers. The effect of ALAN on macroinvertebrate density found after one week, persisted in the third and fourth weeks of exposure and for one further week after the end of the experiment when recolonization by macrofauna occurred in the absence of ALAN. This effect was found only in spring, suggesting that the effect of ALAN might

be dependent on macroinvertebrate phenology. In autumn community composition was markedly different than in spring. Taxa composing the macroinvertebrate community in autumn may be less sensitive to ALAN, or may have a higher propensity to drift, regardless of ALAN exposure. Density was also higher in autumn in both control and treatment flume sections. High densities may have caused an increase in animal drift due to density-dependent effects (competition for space), overriding the drift-inhibiting effect of ALAN that was observed in spring.

The second study investigated whether the effects of ALAN can propagate from aquatic to terrestrial ecosystems. There was a 3-fold increase in the number of emerging aquatic insects in ALAN-treated traps compared to unlit controls. The number of aquatic flying (i.e., adult) insects attracted to lit traps was up to 460-fold higher than in the dark control. The proportion of total insects in ALAN-treated traps that were aquatic was up to 4-fold higher than the dark site. ALAN increased emergence and attraction of insects to the treatment field and changed prey quantity and quality for ground-dwelling secondary consumers. I conclude that, this was the main driver that led to changes in both diurnal and nocturnal ground-dwelling secondary consumer community composition. To test this hypothesis, I conducted the third study of the thesis.

In the third study, using the same ditch experimental field, I used stable isotope analysis to test whether the observed change in prey subsidy dynamics in ALAN-treated riparian areas resulted in a change in the diet of terrestrial arthropod consumers. The carbon isotopic signature of *Pachygnatha clercki* (Tetragnathidae) was 0.7‰ lower in lit site compared to control traps in summer, indicating a greater assimilation of aquatic prey when the large majority of adult insects at lights were aquatic in origin. Bayesian mixing models also showed a 13% increase in aquatic prey intake in summer. In spring, isotopic signatures were more similar to terrestrial prey in lit traps compared to dark traps for *P. clercki* (0.3‰) and *Pardosa prativaga* (0.7‰), despite 80% of prey being aquatic at both sites. Bayesian mixing models showed increased terrestrial prey intake in all three taxa analysed (*P. clercki* and Opiliones 4%, *P. prativaga* 9%). In autumn, mixing models also indicated greater assimilation of terrestrial carbon for *P. prativaga* (5%) and Opiliones (7%) in lit traps, despite there being a higher proportion of aquatic insects at the lit site. In spring and autumn, with lower number of available prey (both, aquatic and terrestrial) compared to summer, or with more similar abundance between aquatic and terrestrial prey, it is likely that

consumers fed more on terrestrial prey with higher biomass (e.g. moths, leaf hoppers) than on the small-sized aquatic component (e.g. mayflies, non-biting midges). These results suggest that the effect of ALAN on the diet of riparian consumers can be dependent on phenological patterns of both consumers and prey.

Without a doubt ALAN has enhanced the human wellbeing by extending economically productive and recreational activities into nocturnal hours and increasing the feeling of safety. However, the transformation of nightscapes is increasingly recognized as harmful for natural ecosystems. Results from my thesis show that ALAN should be considered a relevant ecological stressor in urban and landscape planning and that the illumination of aquatic and riparian ecosystems should be minimised. I found that mayflies are particularly sensitive to ALAN both as larvae (*Baetis* spp.) and adults (*Cloeon* sp.). As they are widespread in freshwater ecosystems and known to be sensitive to environmental degradation, Baetidae are used as bio-indicator. My results suggest that they would also be suitable indicators of ALAN stress in restoration and biomonitoring programs on aquatic and riparian ecosystems. Due to the important role of mayflies in aquatic food webs and, after emerging, as subsidies to consumers in recipient ecosystems, altered abundance of this group of insects might have important implications for top-down or bottom-up food web regulative processes and thus on the ecosystem functioning of both aquatic and their adjacent riparian areas. Increased availability of aquatic prey subsidies in the riparian areas due to ALAN may also have severe consequences for the natural control by predation of invertebrate pest populations (e.g. Aphidae, Auchenorrhyncha) which can threaten agricultural production.

Zusammenfassung

Die Erhellung der Nacht durch künstliches Licht (ALAN, Artificial Light at Night) ist heutzutage eine der am meist verbreiteten anthropogenen Einflussgrößen auf Nachtlandschaften. Zu den Konsequenzen von ALAN gehören unter anderem die Beeinflussung von Verhalten und Migration von zahlreichen Tierarten. Dies kann wiederum zu Veränderungen in den räumlichen und zeitlichen Artverteilungsmustern führen und hat damit potentiell Einfluss auf Räuber-Beute-Beziehungen innerhalb und zwischen verschiedenen Ökosystemen. Es ist anznehmen, dass Gewässer und ufernahe Bereiche in besonderem Maße von den Folgen zunehmender künstlicher Beleuchtung betroffen sind, da diese oft in der Nähe menschlicher Siedlungen oder Aktivitäten liegen. Aquatische Systeme stehen mit den angrenzenden terrestrischen Bereichen in Verbindung. Der Austausch von (organischem) Material und Organismen stellt eine wichtige Quelle für Nährstoffe und Energie für das das jeweilige Empfänger-Ökosystem dar. Jüngste Studien haben gezeigt, dass sich anthropogene Einflüsse auf aquatische Systeme über qualitative und quantitative Veränderungen in der Beschaffenheit der Beutetierpopulationen in die angrenzenden terrestrischen Bereiche übertragen kann. Das Ausmaß der Auswirkung von künstlichem Licht in der Nacht auf solche ökologischen Wechselwirkungen ist jedoch bis heute weitgehend unbekannt.

Im Rahmen dieser Doktorarbeit wurden drei Feldstudien in zwei unterschiedlichen Ökosystemen durchgeführt. Der erste Versuch wurde in künstlich angelegten Fließrinnen an einem sub-alpinen Flusses durchgeführt, und der Einfluss von künstlichem Licht auf ALAN-naive aquatische Makroinvertebratengemeinschaften betrachtet. In einer zweiten Studie an landwirtschaftlichen Drainagegräben wurde untersucht, ob sich die Auswirkungen von ALAN über einen veränderten Eintrag aquatischer Insekten auf die invertebraten Prädatoren und Aasfresser im Uferbereich auswirken. Eine dritte Studie an demselben Grabensystem analysierte den Effekt der lichtinduzierten Veränderung der Beutetierzusammensetzung auf die Ernährungsgewohnheiten der im Uferbereich lebenden invertebraten Prädatoren und Aasfresser.

Die erste Studie zeigte, dass eine einwöchige nächtliche Beleuchtung sowohl Abundanz als auch die taxonomische und funktionelle Zusammensetzung der

benthischen Invertebratengemeinschaften in den Fließrinnen beeinflusste. Nach einer Woche waren Chironomidae und *Baetis* spp. in den Fließrinnen viermal häufiger vertreten als in den Kontollrinnen unter natürlichen Bedingungen. Die Analyse der funktionellen Ernährungstypen ergab, dass Weidegänger in den beleuchteten Fließrinnen 1.5 mal häufiger waren als unter natürlichen Bedingungen, während Filtrierer nur halb so stark vertreten waren. Diese Ergebnisse legen nahe, dass künstliche nächtliche Beleuchtung das Driftverhalten der Chironomidae, *Baetis* spp. und Weidegänger unterdrückt. Die nach einer Woche beobachteten Effekte auf die Populationsdichte von Makroinvertebraten durch künstlich Beleuchtung blieben auch in der dritten und vierten Woche des Experiments hindurch bestehen sowie eine Woche nach dem Experiment bevor dann eine Rekolonialisierung bei natürlicher Dunkelheit zu beobachten war. Dieser Effekt konnte nur im Frühling beobachtet werden, was impliziert, dass die Auswirkung von nächtlicher Beleuchtung von der Phänologie der Makroinvertebraten abhängen kann. Die Zusammensetzung der Artengemeinschaft im Herbst unterschied sich deutlich von der im Frühjahr. Die Taxa der herbstlichen Makroinvertebratengemeinschaft schienen weniger sensitiv auf künstliche Beleuchtung zu reagieren, oder besitzen eine höhere, von künstlicher Beleuchtung unabhängige Neigung zu driften. Zudem waren die Populationsdichten im Herbst in allen Fließrinnen höher. Dies könnte eine dichtabhängige Driftreaktion (Wettbewerb um Lebensraum) ausgelöst haben, welche den im Frühling beobachteten drifthemmenden Effekt der künstlichen Beleuchtung ausgleicht.

Die zweite Studie untersuchte, ob sich die Auswirkungen der künstlichen Beleuchtung von dem aquatischen in das angrenzende terrestrische Ökosystem fortpflanzen kann. In den beleuchteten Emergenzfallen gab es im Vergleich zu den Fallen in den unbeleuchteten Kontrollflächen dreimal so viel emergierende Insekten. Die Anzahl fliegender (d.h. adulter) aquatischer Insekten war an dem beleuchteten Standort bis zu 460-fach erhöht. Dabei war der Anteil aquatischer Insekten an den beleuchteten Standorten viermal höher als an den dunklen. Künstliche Beleuchtung erhöhte somit die Anzahl der emergierenden Insekten sowie die Anziehung von fliegenden Insekten, was wiederum die Qualität und Quantität der Beutetiere für die am Boden lebenden Sekundärkonsumenten verändert hat. Dies war vermutlich der Hauptgrund für die Veränderungen in der Zusammensetzung der tag- sowie nachtaktiven Sekundärkonsumentengemeinschaften. Um diese Hypothese zu testen, wurde im Rahmen dieser Doktorarbeit eine dritte Studie durchgeführt.

In der dritten Studie wurden Signaturen stabiler Isotope untersucht, um zu testen, ob die beobachteten Veränderungen in dem Angebot von potentiellen Beutetieren zu einer Veränderung der Ernährungsgewohnheiten der terrestrischen Konsumenten in den beleuchteten Uferbereichen führt. Die Kohlenstoffisotopensignatur von *Pachygnatha clercki* (Tetragnathidae) war im Sommer an den beleuchteten Standorten 0.7‰ niedriger als an den dunklen Kontrollflächen. Dies deutet darauf hin, dass an den beleuchteten Standorten mit überwiegend aquatischen adulten Insekten auch ein höherer Anteil aquatischer Beutetiere aufgenommen wurde. Auch die Analyse mithilfe von gemischten bayesschen Modellen zeigten im Sommer eine Erhöhung der Nahrungsaufnahme aquatischer Beutetiere um 13%. Im Frühling waren die Isotopensignaturen der Konsumenten an den beleuchteten Standorten denen der terrestrischen Beutetiere ähnlicher als die der Konsumenten an den unbeleuchteten Kontrollstandorten (*P. clercki* mit 0.3‰, *Pardosa prativaga* mit 0.7‰), obwohl 80% der Beutetierpopulationen an beiden Standorten aquatischen Ursprungs waren. Die gemischten bayesschen Modelle zeigten hier zudem eine erhöhte Aufnahme terrestrischer Beutetiere in allen analysierten Taxa (*P. clercki* and Opiliones 4%, *P. prativaga* 9%). Auch im Herbst zeigten die Modelle an den beleuchteten Standorten eine höhere Aufnahme terrestrischen Kohlenstoffs durch *P. prativaga* (5%) und Opiliones (7%), obwohl der Anteil aquatischer Insekten dort ebenfalls höher war als an den Kontrollstandorten. Der Grund für diese jahreszeitlichen Unterschiede könnte darin liegen, dass das Nahrungsangebot an aquatischen und terrestrischen Insekten im Frühjahr und Herbst insgesamt niedriger war als im Sommer und gleichzeitig das Verhältnis aquatischer und terrestrischer Beutetiere ausgewogener war. Dies könnte dazu führen, dass der Anteil terrestrischer Beutetiere mit ihrer höheren Biomasse (z.B. Motten, Grashüpfer) den der vergleichsweise kleinen aquatischen Insekten (z.B. Eintagsfliegen, Zuckmücken) überwiegt. Diese Ergebnisse legen nahe, dass der Effekt von ALAN auf die Ernährungsgewohnheiten der im Uferbereich lebenden Konsumenten von der Phänologie der Konsumenten als auch der Beutetiere abhängt.

Nächtliche Beleuchtung erhöht zweifelsohne die Qualität für verschiedenartige menschliche Aktivitäten, indem sie beispielsweise die Zeiten für ökonomische Produktivität und Freizeitgestaltung bis in die Nacht verlängert und das Sicherheitsempfinden erhöht. Doch der zunehmende Verlust der Nacht wird mehr und mehr als eine Belastung für Ökosysteme betrachtet. Die Ergebnisse dieser

Doktorarbeit zeigen, dass ALAN als relevanter ökologischer Stressfaktor in der Stadt- und Landschaftsplanung berücksichtigt werden muss, und dass die Beleuchtung von Gewässern und Uferbereichen minimiert werden sollte. Eintagsfliegen reagierten sowohl im Larvenstadium (z. B. *Baetis* spp.) als auch im adulten Zustand (z. B. *Cloeo*n sp.) besonders empfindlich auf Lichtverschmutzung. Da sie weitverbreitet und gleichzeitig sensibel gegenüber Umweltverschmutzung sind, sind Baetidae wichtige Bioindikatoren. Die Ergebnisse dieser Studie zeigen, dass Eintagsfliegen auch für die Indikation von Lichtverschmutzung im Rahmen von Gewässersanierungs- oder Biomonitoringprogrammen geeignet sind. Aufgrund ihrer bedeutenden Rolle in aquatischen Nahrungsnetzen als auch als wichtiges Beutetier für Konsumenten in angrenzenden terrestrischen Ökosystemen, kann sich eine veränderte Populationsdichte dieser Insektengruppe stark auf die regulativen Prozesse innerhalb der Nahrungsnetze und damit auf den Zustand aquatischer sowie angrenzender Ökosysteme auswirken. Eine durch künstliche Beleuchtung verursachte höhere Verfügbarkeit aquatischer Beutetiere in ufernahen Bereichen könnte ebenfalls deutliche Folgen für die natürliche Regulation (z.B. durch Prädation) invertebrater Schädlingspopulationen (z.B. Aphidae, Auchenorrhyncha) haben, was zu Beeinträchtigungen in der landwirtschaftlichen Produktion führen kann.

Thesis outline and collaboration statement

This thesis is composed of a general introduction that provides the background of the thesis. Three manuscripts that are under revision or are ready to be submitted to peer-reviewed journals form the three central chapters. Each manuscript is meant to stand alone and therefore contains an abstract, introduction, material and methods, results and discussion. References for each chapter are given at the end of that chapter. The thesis concludes with a general discussion chapter. The thesis aims are described in Paragraph 1.5 of the general introduction and repeated, together with a thesis rationale, in Paragraph 5.1 of the general discussion.

Chapter 1: General introduction

Chapter 2: Manfrin A., Bruno M. C., Grubisic M., Monaghan M. T., Hölker F. (*manuscript in preparation*). Artificial light at night (ALAN) affects structural and functional aspects of macroinvertebrate assemblages: a field experiment in a previously ALAN-naïve area.

Author contributions:
All authors designed the study. A. Manfrin, M. C. Bruno and M. Grubisic organized and performed field and laboratory work. A. Manfrin analysed the data. All authors, M. T. Monaghan and F. Hölker contributed to the final manuscript.

Chapter 3: Manfrin A., Larsen S., Weiß N., van Grunsven R. H. A., Weiß N-S., Wohlfahrt S., Singer G., Monaghan M. T., Hölker F. (*manuscript in preparation*). Artificial light at night alters flux across ecosystem boundaries and community structure in the recipient ecosystem.

Author contributions:
All authors designed the study. A. Manfrin, N. Weiß, N-S. Weiß and S. Wohlfahrt organized and performed field and laboratory work. A. Manfrin, S. Larsen, R. H. A. van Grunsven and G. Singer contributed to the data analysis. All authors, M. T. Monaghan and F. Hölker contributed to the final manuscript.

Chapter 4: Manfrin A., Lehmann D., van Grunsven R. H. A., Larsen S., Syväranta J., Wharton G., Voigt C. C., Monaghan M. T., Hölker F. (*manuscript submitted to Oikos and under review*). Dietary changes in predators and scavengers in a riparian ecosystem illuminated at night.

Author contributions:

A. Manfrin, C. C. Voigt, M. T. Monaghan and F. Hölker designed the study. A. Manfrin organized and performed field and laboratory work. A. Manfrin, D. Lehmann and J. Syväranta contributed to the data analysis. All authors, R. H. A. van Grunsven, S. Larsen and G. Wharton contributed to the final manuscript.

Chapter 5: General discussion

1. General introduction

1.1 Artificial light at night (ALAN)

Since the beginning of human civilization, people have been looking at the starry sky at night as source of inspiration. Egyptians, Mayans, Chinese and many others shaped their cultural systems by observing and studying the dark sky. Many gods and goddesses were seen in planets or constellations and stars were used to navigate and to explore new lands (Brecher and Feirtag 1981, Hadingham 1985). A dark night sky was fundamental to relate astronomical patterns to natural patterns regulating life on Earth. The advent of the industrial civilization, followed by urbanisation and economic development over recent decades, led to increased density and distribution of artificial illumination worldwide. (Fig. 1a) (Riegel 1973, Holden 1992, Cinzano et al. 2001, Cinzano 2003, Hölker et al. 2010, Gaston et al. 2013).

Artificial light at night (ALAN) was initially identified as a problem by astronomers because human-induced light pollution of the nocturnal sky caused disturbance in observing stars and celestial bodies (Fig. 1b, c) (Riegel 1973, Longcore and Rich 2004). On the other hand, artificial illumination enhanced the quality of human life (Jakle 2001, Doll et al. 2006). With the advent of artificial light, human productivity was extended into nocturnal hours as the night no longer meant the end of activity. Building exteriors were lit for aesthetic purposes and shopping malls were decorated with coloured lights to attract people and stimulate them to spend (Mower et al. 2012). In urban areas, light levels have been set high as a deterrent against crime (Falchi et al. 2011). For all these reasons, artificial illumination has been associated with a feeling of safety and progress (Perkin et al. 2011). Only recently have the implications of ALAN on ecology, human health and social aspects been considered (Rich and Longcore 2006, Navara and Nelson 2007, Hölker et al. 2010, Gaston et al. 2013).

Different sources of artificial illumination contribute to increase illumination levels in the sky. Some sources of direct artificial illumination include streetlights, illuminated buildings, security lights, fishing boat lights, (see Longcore and Rich 2004, Hölker et al. 2010, Gaston et al. 2013). Over larger areas, direct illumination can be scattered back from the lower layer of the atmosphere and form a lower intensity background illumination known as “sky glow” (Kyba et al. 2011) (Fig. 1b, c).

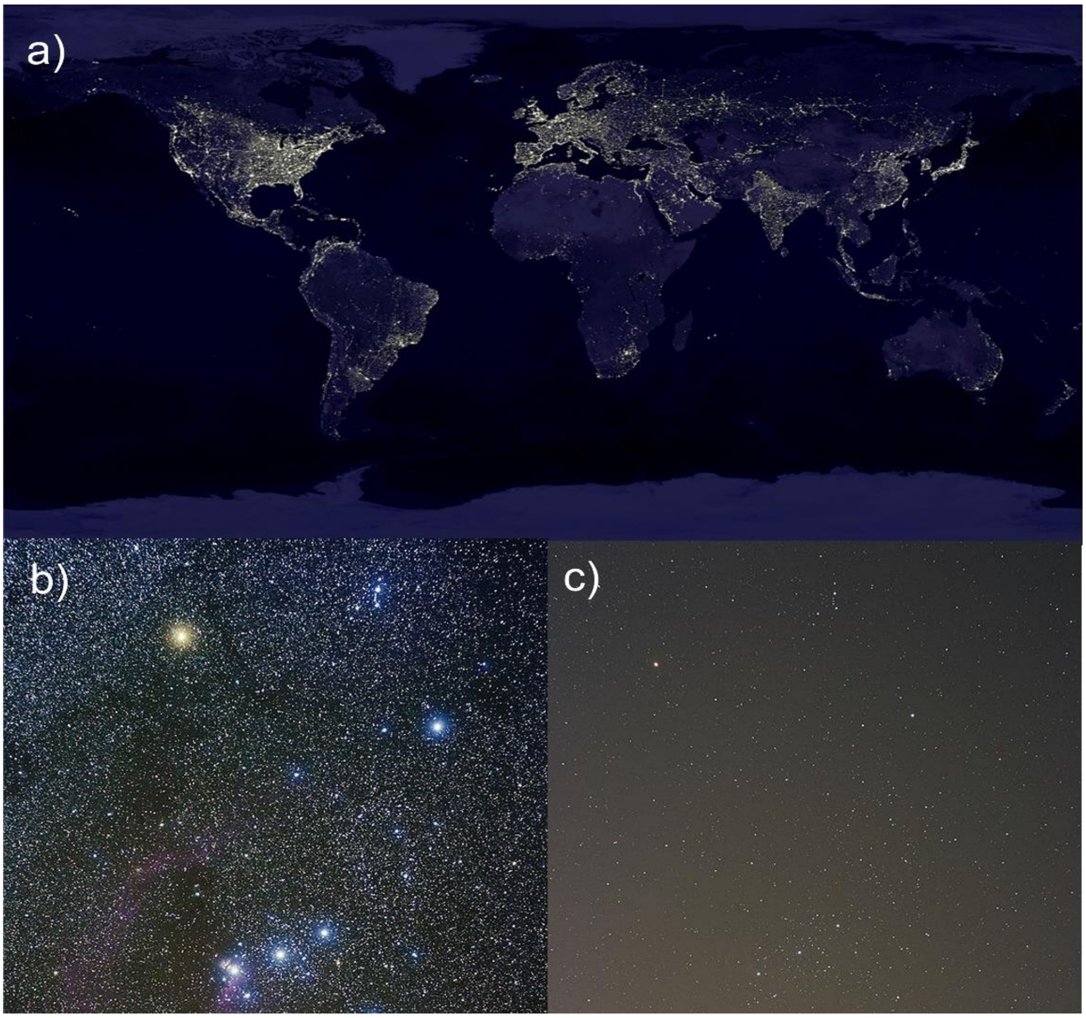


Figure 1. Artificial light at night is widespread throughout the world (a) (Screenshot by NASA’s EOSDIS Worldview: http:// earthdata.nasa.gov/ labs/worldview/, taken on 09 November 2016). Direct illumination can be scattered back from the atmosphere forming a low intensity background illumination known as “sky glow”. This can reduce contrast in the night sky causing disturbance in observing stars and celestial bodies (b, c; photos by Jeremy Stanley).

ALAN is mostly considered in respect to human vision. However, many biological processes (e.g. photosynthesis, circadian clocks) are more sensitive to specific parts of the light spectrum and therefore can be differentially affected by different light sources as these differ in spectral composition (Fig. 2) (Elvidge et al. 2010, Gaston et al. 2013). For instance, Low-pressure sodium (LPS) lamps are restricted to very narrow bandwidths emitting a single peak at 589 nm. More common lighting technologies used today for the majority of streetlights, emit over broader wavelengths. High-pressure sodium (HPS) lamps emit over the yellow spectral component, while light-emitting diode (LED) lamps typically emit at all wavelengths between around 400 and 700 nm, with peaks in the blue and green (see Fig. 2) (Elvidge et al. 2010, Gaston et al. 2013).

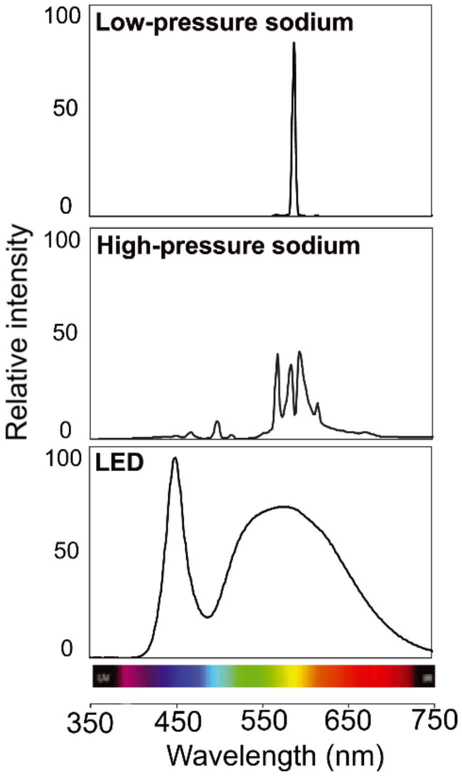


Figure 2. Spectral composition of three common lighting types. Data from Gaston et al. (2013).

1.2 Effect of ALAN on organisms

Many biological patterns in wild organisms are regulated by natural light/dark cycles (Hölker et al. 2010, Gaston et al. 2013). Diel day/night cycles are determined by the rotation of the Earth around its axis while annual planetary orbit determines the length of day and night in each season. These light/dark cycles have been extremely consistent for long geological eras within each latitude allowing organisms to time their daily and annual behaviour (Ragni and D'Alcala 2004, Bradshaw and Holzapfel 2010).

Light/dark patterns regulate circadian and circannual cycles of activity in many organisms. This includes daily timing such as dawn song in birds (Da Silva et al. 2017) but also seasonal phenological events such as plant flowering (Searle and Coupland 2004), animal reproduction (e.g. Nelson 1985, Ciereszko 1997) and insect development (e.g. larval growth, emergence) (e.g. Corbet 1964, Nisimura et al. 2001).

Alterations of natural light/dark cycles due to ALAN have been shown to have several effects on animals (reviewed in Longcore and Rich 2004, Rich and Longcore 2006, Navara and Nelson 2007, Bruce-White and Shardlow 2011). ALAN has been observed to affect animal orientation (e.g. Peters and Verhoeven 1994, Moore et al. 2001, Lorne and Salmon 2007, Stone et al. 2009), dispersal (e. g. Eisenbeis et al. 2006, Degen et al. 2016), foraging (e. G. Rydell 1991, Buchanan 1993, Negro et al. 2000, Bird et al. 2004, Tabor et al. 2004, Santos et al. 2010), interspecific interactions (e. g. Svensson and Rydell 1998), communication (e. g. van Geffen et al. 2015b, Baker and Richardson 2006, Miller 2006) and reproduction (Boldogh et al. 2007, van Geffen et al. 2015a). In many cases ALAN has been observed to contribute directly to organism mortality (Dick and Donaldson 1978, Peters and Verhoeven 1994, Le Corre et al. 2002, Black 2005).

The effect of ALAN can be particularly strong in nocturnal animals which include 30% of all vertebrates and more than 60% of the invertebrates (Hölker et al. 2010). These animals have evolved to be active and foraging in the dark. Under natural light regimes, the highest light level that they experience can reach 0.3 lux at full moon in open habitats. Many groups of nocturnal animals including fish, spiders and insects show attraction to light (positive phototaxy) (Haymes et al. 1984, Nakamura and Yamashita 1997, Summers 1997, Munday et al. 1998, Eisenbeis 2006). Not only nocturnal but also diurnal species can be affected by ALAN. An extension of the lit phase during the night due to ALAN might extend the animal's diurnal activity into the night. This might lead to ecological overlap between diurnal and nocturnal communities with unknown consequences for interspecific interactions (e.g. competition, predator-prey relations).

1.3 Effect of ALAN on the coupled aquatic-terrestrial ecosystems

ALAN is particularly widespread near freshwater ecosystems where human populations are concentrated (see Fig. 3). Approximately half of the world's population is concentrated within 3 km distance to freshwater bodies (Kummu et al. 2011). These are a source of drinking water and food, and are used for transport and recreation (Kummu et al. 2011). Inland waters (e.g. streams, lakes, ponds) are also “hot spots” of biodiversity with 10% of all known animal species although they cover less than 1% of the Earth’s surface. In such environments, water surface light levels at night can range between 4 and 17 lux (Meyer and Sullivan 2013, Perkin et al. 2014a, b, Hölker et al. 2015). This is significantly higher than 0.3 lux of the highest natural light level at night, the full moon. Understanding the effect of ALAN on these ecosystems is thus crucial, considering that freshwater habitats are widely recognized as the most threatened on Earth (Vörösmarty et al. 2010, Dijkstra et al. 2014). ALAN can disrupt natural behavioural patterns such as diel vertical migration of zooplankton and macroinvertebrate drift (Bishop 1969, Moore et al. 2001, Perkin et al. 2014b) and can alter fish predatory efficiency (Tabor et al. 2004) and migration (Cullen and McCarthy 2000, Hansen and Jonsson 1985). However, despite clear evidence of the detrimental effects of ALAN on freshwater ecosystems, knowledge gaps remain (Perkin et al. 2011).

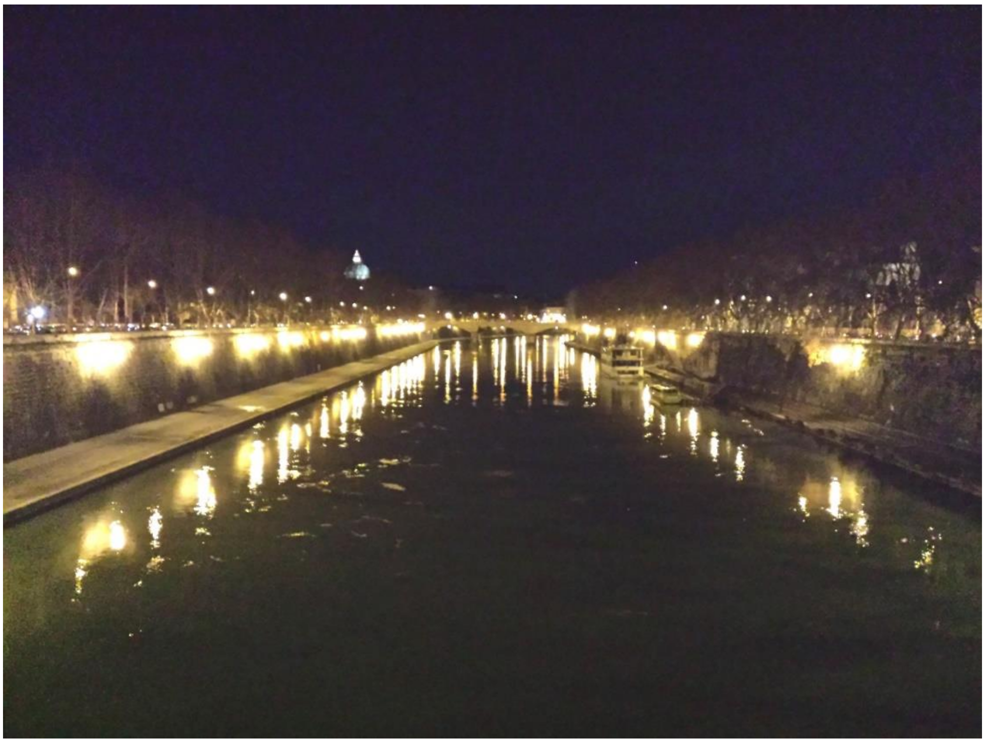


Figure 3. Example of illumination alongside the Tever river in Rome, Italy (Photo by Alessandro Manfrin).

Freshwater bodies are not ecologically isolated ecosystems, but are tightly connected to their adjacent terrestrial ecosystems by fluxes of nutrients, organic matter and organisms. These subsidy fluxes can be reciprocal, benefitting consumers in either of these contiguous habitats (Polis et al. 1997, Nakano et al. 1999, Nakano and Murakami 2001, Richardson et al. 2010) and contributing to the overall food-web and ecosystem stability (Takimoto et al. 2002). Terrestrial-derived carbon and nutrients, often in the form of plant matter, support aquatic metabolism (Wallace et al. 1999, Mehner et al. 2005) while aquatic-derived carbon in the form of fish and aquatic insects are among the most important aquatic prey subsidies for a wide range of consumers inhabiting terrestrial riparian areas (Fig. 4a) (Marczak and Richardson 2007, Bartels et al. 2012).

Aquatic insects are organisms with at least one stage of the life cycle that is aquatic, and most aquatic insects have one or more terrestrial stages. Species with both aquatic and terrestrial stages typically have aquatic eggs and immatures (larvae or nymphs) and terrestrial adults. Because most of the biomass is formed in the immature stages and is derived from aquatic carbon sources, upon emergence these insects form an important flux of aquatically derived carbon and nutrients to the terrestrial ecosystem. They play a large diversity of ecological roles in both realms as primary consumers, detritivores, predators, and pollinators. The number of species of aquatic insects is estimated at more than 200,000 (80% of aquatic animal diversity) (Dijkstra et al. 2014).

Previous studies have found that ALAN can attract aquatic insects to terrestrial ecosystems, disrupt natural inland dispersal patterns and increase mortality by exhaustion (Horváth et al. 2009, Perkin et al. 2014a). These studies raise the possibility that ALAN can disturb the natural subsidies between aquatic and terrestrial ecosystems (Meyer and Sullivan 2013). However, our understanding of the effects of ALAN on the linkage between freshwater ecosystems and adjacent terrestrial ecosystems remains limited (Fig 4b).

1.4 Knowledge gaps

Despite the increasing amount of research on the ecological impact of ALAN in recent decades (Longcore and Rich 2004, Hölker et al. 2010, Gaston et al. 2015) many knowledge gaps remain. Most studies have been performed in terrestrial ecosystems while only a few have considered aquatic ecosystems (e.g. see Perkin et al. 2011, 2014b, Hölker et al. 2015). Even less is known about how ALAN can affect the coupled aquatic-terrestrial ecosystem linkage (but see Meyer and Sullivan 2013). While most studies have investigated the effect of ALAN on individual species (reviewed in Longcore and Rich 2006, Navara and Nelson 2007, Bruce-White and Shardlow 2011) (see Paragraph 1.3), few have analysed its effect on communities and ecosystem functioning (e.g. Hölker et al. 2015, Spoelstra et al. 2015). In freshwater systems, ALAN was found to inhibit drifting behaviour of benthic invertebrates (Bishop 1969, Perkin et al. 2014b), but whether ALAN can change macroinvertebrate taxonomical and functional community composition is poorly investigated (Perkin et al. 2014b). ALAN has been shown to attract post-emerging aquatic insects to terrestrial areas (Perkin et al. 2014a); however, the effect of ALAN on insect emergence is still largely unknown (Meyer and Sullivan 2013). Although we know that ALAN can attract aquatic insects to terrestrial ecosystems, how this affects species composition and diet of riparian secondary consumer communities (e.g spiders and ground-beetles) through changes in aquatic prey availability is unknown. Many studies have investigated the effect of ALAN using pre-existing streetlights. In these studies, the effect of ALAN could not be separated from other aspects of urbanisation which can be confounded with the effects of ALAN. Finally, investigations of ecosystems that have long been exposed to artificial illumination might miss the initial impact due to adaptation to ALAN (but see Bennie et al. 2015, Hölker et al. 2015, Spoelstra et al. 2015).

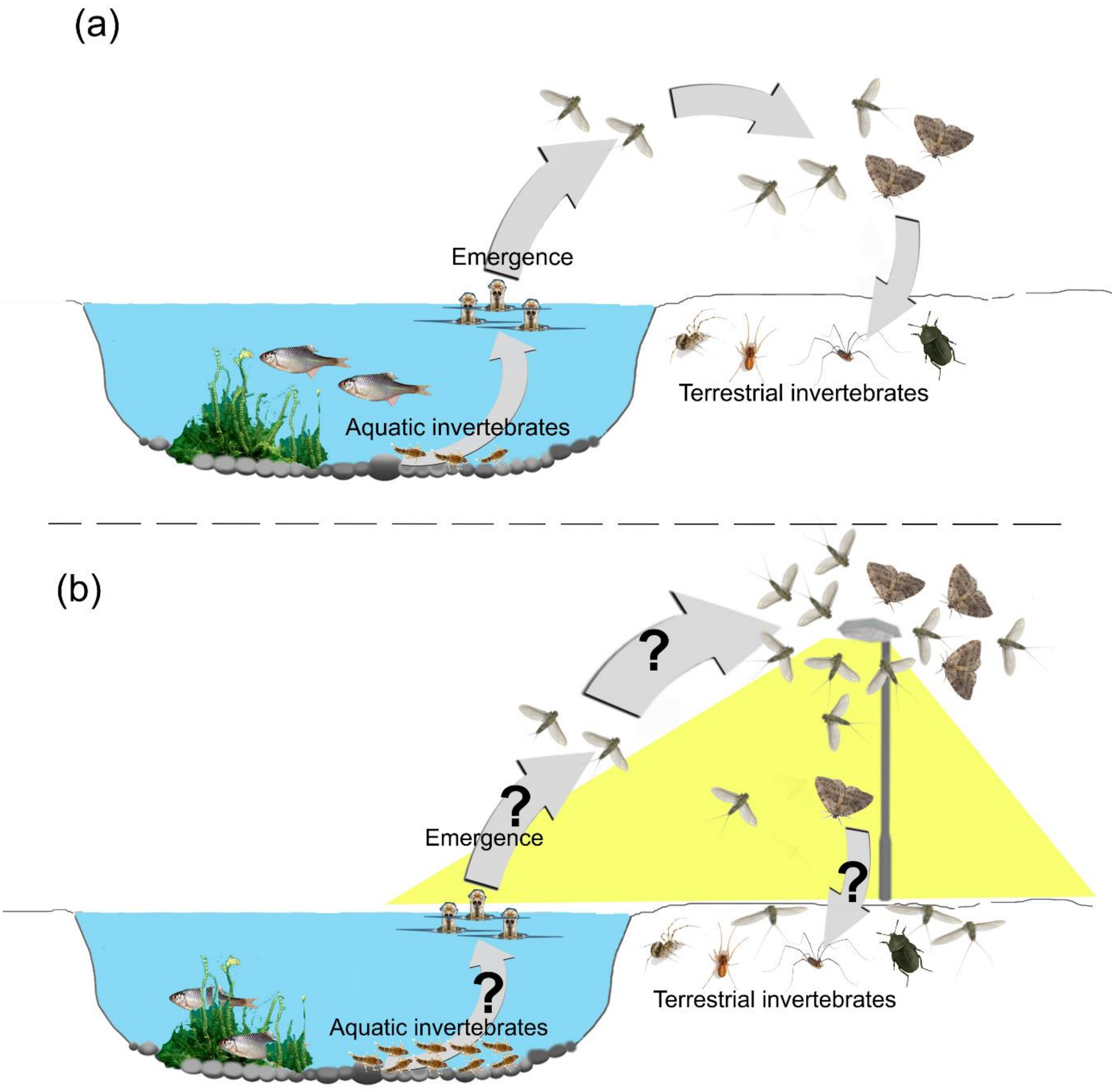


Figure 4. Aquatic and terrestrial ecosystems are tightly connected by fluxes of organic matter and organisms. Post-emerging aquatic insects are a cross-habitat linkage between donor-aquatic and recipient-terrestrial ecosystems being an important subsidy to terrestrial consumers (e.g. spiders, beetles) (a). ALAN has been shown to reduce macroinvertebrate drift and to attract adult flying insects. However, the quality and quantity of the effect of ALAN on the aquatic-terrestrial linkage has been scarcely investigated leaving many questions unanswered (b).

1.5 Thesis aims and approach

I investigated the effect of ALAN on aquatic invertebrate communities experimentally exposed to artificial illumination in two field experiments. A first experiment (Chapter 2) was performed in a flume system fed by a pristine sub-alpine stream (Fig. 5a, b) and a second experiment (Chapters 3 and 4) was conducted in an experimental set-up in an agricultural drainage ditch system (Fig. 5c, d).

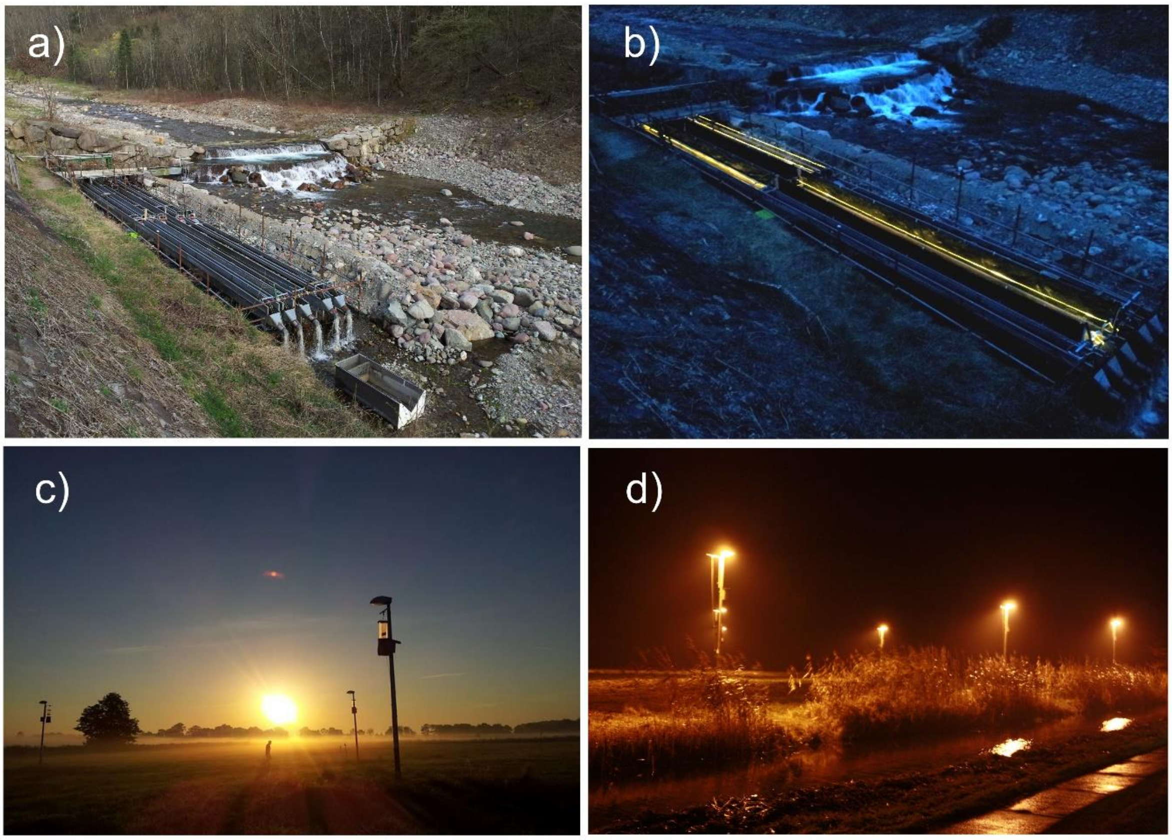


Figure 5. In Chapter 2 a set of five metal flumes installed on the right bank of a pristine sub-alpine stream in northern Italy (a) were artificially illuminated during the night (b) (further details are given in Chapter 2). In Chapters 3 and 4 streetlights were used to experimentally illuminate an agricultural drainage ditch in northern Germany (c, d) (further details in Chapters 3 and 4) (Photos by Alessandro Manfrin).

Chapter 2 aimed to assess the effect of ALAN on density and composition of riverine macroinvertebrate communities. I hypothesized that ALAN will induce taxon-specific responses (e.g. decreased drift) in macroinvertebrate communities, depending on phototaxic response of the taxa. This would lead to changes in community structure and function. To test this hypothesis, I measured the effects of ALAN on density and on taxonomic and functional composition of benthic macroinvertebrate communities over four weeks in both spring and autumn. After the first week, I assessed the effect of ALAN on ALAN-naïve communities. After the third week, I assessed the effect of ALAN on communities that had already been exposed for 2 weeks. I also measured changes in communities after returning to natural light/dark regimes for a week after exposure to ALAN for 3 weeks. For this study light-emitting diode (LED) lights were installed on a set of 5 experimental flumes fed by a sub-alpine stream in northern Italy (Fig. 5a, b).

In Chapter 3 I aimed to investigate the effect of illumination on (i) aquatic insect emergence, considered as a primary source of aquatic subsidies to the terrestrial system; (ii) the spatial and temporal distribution of flying aquatic and terrestrial insects in the riparian environment; and (iii) the abundance and composition of riparian ground-dwelling predator and scavenger communities. I hypothesized that ALAN attracts aquatic and terrestrial insects affecting their spatial and temporal distribution. This in turn would affect ground-dwelling predator and scavenger communities in response to the light-induced changes in prey availability. I assessed the effect of ALAN on natural dynamics of aquatic insect fluxes (as emerging and flying adults) from a donor aquatic to a recipient terrestrial ecosystem using emergence and air eclector traps. In the adjacent terrestrial ecosystem, communities of terrestrial arthropod consumers, feeding on those subsidies, were investigated using pitfall traps. This study was carried out over 2 years using a large-scale experimental infrastructure located in Westhavelland Nature Park, one of the darkest area in northern Germany. Lamp posts were installed in grasslands in proximity of 2 drainage ditches: one was illuminated using high-pressure sodium (HPS) lights and used as ALAN-treated site and the other one was used as dark control (Fig. 5c, d).

In Chapter 4, using the same experimental setup used in Chapter 3, I further explored whether ALAN changes the diet composition of ground-dwelling secondary consumers in the riparian areas. I hypothesized that increased aquatic prey subsidies caused by ALAN would result in increased consumption of aquatic prey by riparian secondary consumers, changing their dietary composition. This was tested using stable isotope analysis of riparian secondary consumers and their aquatic and terrestrial prey species over spring, summer and fall.

Together these chapters give insight in how ALAN affects macroinvertebrates in aquatic systems and how ALAN affects the linkage between aquatic and riparian ecosystems in terms of the movement of animals between these systems and their subsequent impact on the food web.

1.6 References

Baker, B. J., and J. M. L. Richardson. 2006. The effect of artificial light on male breeding-season behaviour in green frogs, *Rana clamitans melanota*. Canadian Journal of Zoology **84**: 1528-1532.

Bartels, P., J. Cucherousset, K. Steger, P. Eklöv, L. J. Tranvik, and H. Hillebrand. 2012. Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer resource dynamics. Ecology **93**: 1173-1182.

Bennie, J., T. W. Davies, D. Cruse, R. Inger, and K. J. Gaston 2015. Cascading effects of artificial light at night: resource-mediated control of herbivores in a grassland ecosystem. Philosophical. Transactions of the Royal Society B **370**, 20140131.

Bird, B. L., L. C. Branch, and D. L. Miller. 2004. Effects of coastal lighting on foraging behavior of beach mice. Conservation Biology **18**: 1435-1439.

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

Black, A. 2005. Light induced seabird mortality on vessels operating in the Southern Ocean: incidents and mitigation measures. Antarctic Science **17**: 67-68.

Boldogh, S., D. Dobrosi, and P. Samu. 2007. The effects of the illumination of buildings on house-dwelling bats and its conservation consequences. Acta Chiropterologica **9**: 527-534.

Bradshaw, W. E., and C. M. Holzapfel. 2010. Light, time, and the physiology of biotic response to rapid climate change in animals. Annual Review of Physiology **72**: 147-166.

Brecher, K., and M. Feirtag 1979. Astronomy of the ancients. Cambridge, MA, MIT Press.

Bruce-White, C., and M. Shardlow. 2011. A Review of the impact of artificial light on invertebrates. Buglife, Peterborough

Buchanan, B. W. 1993. Effects of enhanced lighting on the behaviour of nocturnal frogs. Animal Behavior **45**: 893-899.

Ciereszko, R. E., K. Dabrowskl, A. Ciereszko, J. Ebeling, and J. S. Ottobre. 1997. Effects of temperature and photoperiod on reproduction of female yellow perch *Perca flavescens*: plasma concentrations of steroid hormones, spontaneous and induced ovulation, and quality of eggs. Journal of the World Aquaculture Society **28**: 344-356.

Cinzano, P. 2003. The growth of the artificial night sky brightness over North America in the period 1947–2000: a preliminary picture. Pages 39-47 *in* Schwarz H. E. editors. Light Pollution: The Global View. Kluwer Academic, Dordrecht.

Cinzano, P., F. Falchi and C. D. Elvidge. 2001. The first World atlas of the artificial night sky brightness. Monthly Notices of the Royal Astronomical Society **328**: 689-707.

Corbet, P. S. 1964. Temporal patterns of emergence in aquatic insects. The Canadian Entomologist **96**: 264-279.

Cullen, P., and T. K. McCarthy. 2000. The Effects of Artificial Light on the Distribution of Catches of *Silver Eel*, *Anguilla anguilla* (L.), across the Killaloe Eel Weir in the Lower River Shannon. Biology and Environment: Proceedings of the Royal Irish Academy **100**: 165-169.

Da Silva, A., M. de Jong, R. H. van Grunsven, M. E. Visser, B. Kempenaers, and K. Spoelstra. 2017. Experimental illumination of a forest: no effects of lights of different colours on the onset of the dawn chorus in songbirds. Royal Society Open Science **4**, 160638.

Degen, T., O. Mitesser, E. K. Perkin, N. S. Weiß, M. Oehlert, E. Mattig, and F. Hölker. 2016. Street lighting: sex-independent impacts on moth movement. Journal of Animal Ecology **85**: 1352-1360.

Dick, M.H., and W. Donaldson. 1978. Fishing vessel endangered by crested auklet landings. Condor **80**: 235-236.

Dijkstra, K. D. B., M. T. Monaghan, and S. U. Pauls. 2014. Freshwater Biodiversity and Insect Diversification. Annual review of entomology **59**: 143-163

Doll, C. N., J. P. Muller, and J. G. Morley. 2006. Mapping regional economic activity from night-time light satellite imagery. Ecological Economics **57**: 75-92.

Eisenbeis, G., C. Rich, and T. Longcore. 2006. Artificial night lighting and insects: attraction of insects to streetlamps in a rural setting in Germany. Pages 191-198 *in* Rich G. C. and Longcore T. editors. Ecological consequences of artificial night lighting. Island Press, Washington DC, USA.

Elvidge, C. D., D. M. Keith, B. T. Tuttle, and K. E. Baugh. 2010. Spectral identification of lighting type and character. Sensors **10**: 3961–3988.

Falchi, F., P. Cinzano, C. D. Elvidge, D. M. Keith, and A. Haim. 2011. Limiting the impact of light pollution on human health, environment and stellar visibility. Journal of Environmental Management **92**: 2714-2722.

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

Gaston, K. J., M. E. Visser, and F. Hölker. 2015. The biological impacts of artificial light at night: the research challenge. Philosophical Transactions of the Royal Society B **370,** 20140133*.*

Hadingham, E. 1985. Early man and the cosmos. University of Oklahoma Press.

Hansen, L. P. and B. Jonsson. 1985. Downstream migration of hatchery-reared smolts of Atlantic salmon (*Salmo salar L*.) in the River Imsa, Norway. Aquaculture **45**: 237-248.

Haymes, G. T., P. H. Patrick, and L. J. Onisto. 1984. Attraction of fish to mercury vapour light and its application in a generating station forebay. Internationale Revue der gesamten Hydrobiologie und Hydrographie **69**: 867-876.

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

Hölker, F., C. Wurzbacher, C. Weißenborn, M. T. Monaghan, S. I. Holzhauer, and K. Premke. 2015. Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. Philosophical Transactions of the Royal Society B **370**, 20140130.

Holden, A. 1992. Lighting the night: technology, urban life and the evolution of street lighting. Places **8**: 56-63.

Horváth, G., G. Kriska, P. Malik, and B. Robertson. 2009. Polarized light pollution: a new kind of ecological photopollution. Frontiers in Ecology and the Environment **7**: 317-325.

Jakle, J. A. 2001. City Lights: Illuminating the American Night. Johns Hopkins University Press, Baltimore.

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.

Kummu, M., H. De 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**: 1-13.

Le Corre, M., A. Ollivier, S. Ribes, and P. Jouventin. 2002. Light-induced mortality of petrels: a 4-year study from Reunion Island (Indian Ocean). Biological Conservation **105**: 93–102.

Longcore, T., and C. Rich. 2004. Ecological light pollution. Frontiers in Ecology and the Environment **2**: 191-198.

Lorne, J. K., and M. Salmon. 2007. Effects of exposure to artificial lightin on orientation of hatchling sea turtles on the beach and in the ocean. Endangered Species Research **3**: 23–30.

Marczak, L. B., and J. S. Richardson. 2007. Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. Journal of Animal Ecology **76**: 687-694.

Mehner, T., J. Ihlau, H. Dörner, and F. Hölker. 2005. Can feeding of fish on terrestrial insects subsidize the nutrient pool of lakes? Limnology and Oceanography **50**: 2022-2031.

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

Miller, M.W. 2006. Apparent effects of light pollution on singing behavior of American robins. The Condor **108**: 130–139.

Moore, M. V., S. M. Pierce, H. M. Walsh, S. K. Kvalvik, and J. D. Lim. 2001. Urban light pollution alters the diel vertical migration of Daphnia. Internationale Vereinigung fur Theoretische und Angewandte Limnologie Verhandlungen **27**: 779-782.

Mower, J. M., M. Kim, and M. L. Childs. 2012. Exterior atmospherics and consumer behavior: influence of landscaping and window display. Journal of Fashion Marketing and Management: An International Journal **16**: 442-453.

Munday, P. L., G. P. Jones, M. S. Pratchett, and A. J. Williams. 2008. Climate change and the future for coral reef fishes. Fish and Fisheries **9**: 261-285.

Nakamura, T., and S. Yamashita. 1997. Phototactic behavior of nocturnal and diurnal spiders: negative and positive phototaxes. Zoological Science **14**: 199-203.

Nakano, S., H. Miyasaka, and N. Kuhara. 1999. Terrestrial–aquatic linkages: riparian arthropod inputs alter trophic cascades in a stream food web. Ecology, **80**: 2435-2441.

Nakano, S., and M. Murakami. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Sciences **98**: 166-170.

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

Negro, J. J., J. Bustamante, C. Melguizo, J. L. Ruiz, and J. M. Grande. 2000. Nocturnal activity of lesser kestrels under artificial lighting conditions in Seville, Spain. Journal of Raptor Research **34**: 327-329.

Nelson, R. J. 1985. Photoperiod influences reproduction in the prairie vole (Microtus ochrogaster). Biology of Reproduction **33**: 596-602.

Nisimura, T., and H. Numata. 2001. Endogenous timing mechanism controlling the circannual pupation rhythm of the varied carpet beetle *Anthrenus verbasci*. Journal of Comparative Physiology A **187**: 433-440.

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. Freshwater Biology **59**: 368-377.

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

Peters, A., and K. J. F. Verhoeven. 1994. Impact of artificial lighting on the seaward orientation of hatchling loggerhead turtles. Journal of Herpetology **28**: 112–114.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**: 289-316.

Ragni, M., and M. R. D'Alcala´. 2004. Light as an information carrier underwater. Journal of Plankton Research **26**: 433–443.

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

Richardson, J. S., Y. Zhang, and L. B. Marczak. 2010. Resource subsidies across the land–freshwater interface and responses in recipient communities. River Research and Applications **26**: 55-66.

Riegel, K. W. 1973. Light pollution. Science **179**: 1285-1291.

Rydell, J. 1991. Seasonal use of illuminated areas by foraging northern bats Eptesicus nilssoni. Holarctic Ecology **14**: 203-207.

Santos, C. D., A. C. Miranda, J. P. Granadeiro, P. M Lourenco, S. Saraiva, S., and J. M. Palmeirim. 2010. Effects of artificial illumination on the nocturnal foraging of waders. Acta Oecologica **36**: 166-172.

Searle, I., and G. Coupland. 2004. Induction of flowering by seasonal changes in photoperiod. The EMBO Journal **23**: 1217-1222.

Spoelstra, K., R. H. A. van Grunsven, M. Donners, P. Gienapp, M. E. Huigens, R. Slaterus, F. Berendse, M. E. Visser, and E. Veenendaal. 2015. Experimental illumination of natural habitat—an experimental set-up to assess the direct and indirect ecological consequences of artificial light of different spectral composition. Philosophical Transactions of the Royal Society of London B: Biological Sciences **370**, 20140129.

Stone, E. L., G. Jones, and S. Harris. 2009. Street lighting disturbs commuting bats. Current Biology **19**: 1123–1127.

Summers, C. G. 1997. Phototactic behavior of *Bemisia argentifolii* (Homoptera: Aleyrodidae) crawlers. Annals of the Entomological Society of America **90**: 372-379.

Svensson, M. G. E., J. Rydell, and R. Brown. 1999. Bat predation and flight timing of winter moths, Epiritta and Operophtera species (Lepidoptera, Geometridae). Oikos **84**: 193-198.

Tabor, R. A., G. S. Brown, and V. T. Luiting. 2004. The effect of light intensity on sockeye salmon fry migratory behavior and predation by cottids in the Cedar River, Washington. North American Journal of Fisheries Management **24**: 128-145.

Takimoto, G., T. Iwata, and M. Murakami. 2002. Seasonal subsidy stabilizes food web dynamics: balance in a heterogeneous landscape. Ecological Research **17**: 433-439.

van Geffen, K. G., E. Eck, R. A. Boer, R. H. A. van Grunsven, L. Salis, F. Berendse, and E. M. Veenendaal. 2015a. Artificial light at night inhibits mating in a Geometrid moth. Insect Conservation and Diversity **8**: 282-287.

van Geffen, K. G., A. T. Groot, R. H. A. van Grunsven, M. Donners, F. Berendse, and E. M. Veenendaal. 2015b. Artificial night lighting disrupts sex pheromone in a noctuid moth. Ecological Entomology **40**: 401-408.

Vörösmarty, C.J., P. B. McIntyre, M. O. Gessner, D. Dudgeon, A. Prusevich, P. Green, S. Glidden, S. E. Bunn, C. A. Sullivan, C. R. Liermann, and P. M. Davies. 2010. Global threats to human water security and river biodiversity. Nature **467**: 555-561.

Wallace, J. B., S. Eggert, J. L. Meyer, and J. Webster. 1999. Effects of resource limitation on a detrital-based ecosystem. Ecological Monographs **69**: 409-442.

2. Artificial light at night (ALAN) affects structural and functional aspects of macroinvertebrate assemblages: a field experiment in a previously ALAN-naïve area

Manfrin A.[1, 2, 3], Bruno M. C.[4], Grubisic M.[1, 2], Monaghan M. T.[1*], Hölker F.[1*]

[1] Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

[2]Department of Biology-Chemistry-Pharmacy, Freie Universität Berlin, Berlin, Germany

[3]School of Geography, Queen Mary University of London, London, England, UK

[4] Research and Innovation Centre, Fondazione E. Mach, S. Michele all'Adige, Italy

* Authors contributed equally

2.1 Abstract

The area of the Earth’s surface exposed to artificial light at night (ALAN) is increasing worldwide. The use of ALAN is widespread near freshwater bodies, where human populations are concentrated. Light intensities as low as 10^{-3} lux can reduce macroinvertebrate drift in streams, with intensities between 0.1 and 1 lux being enough to entirely suppress it. Light intensities at the water surface of ALAN-exposed streams can exceed these observed thresholds, potentially disrupting diel behaviour patterns in organisms regulated by natural light/dark cycles.

We applied ALAN of an intensity comparable to that commonly found in lit urban and sub-urban areas (*ca*. 20 lux) in a set of sub-alpine streamside flumes. We compared density as well as taxonomic and functional composition of macroinvertebrate communities exposed to ALAN with control communities experiencing natural light/dark cycles. We examined both ALAN-naïve and ALAN-exposed communities, and tested whether control and treatment communities returned to similar densities and composition 1 week after ALAN was removed.

There was a 3-fold increase in macroinvertebrate density in ALAN-treated flume sections after 1 week in spring that we attributed to inhibited drift of *Baetis* and Chironomidae in lit sections. In contrast, density of filter-feeders decreased under ALAN. These effects persisted into the third and fourth weeks of exposure, and 1 week after ALAN was removed and macroinvertebrates were allowed to recolonize. There was no ALAN effect in autumn, when densities and drift rates were much higher, suggesting that effects are dependent on season and macroinvertebrate phenology.

Given the important ecological role of macroinvertebrates in streams, results from our study indicate that functionality of freshwater ecosystems can be substantially impacted by ALAN. Streams are typically exposed to ALAN for long periods of time, and the effect of ALAN might be more pronounced than the short-term effects observed here. These might include impairments of competitive relationships among species or effects on food-web regulatory processes.

2.2 Introduction

Artificial light at night (ALAN) is a prominent feature in many areas and global light emissions are increasing at a rate of up to 20% per year (Narisada and Schreuder 2004, Hölker et al. 2010). ALAN can have multiple effects on organisms, exerting reproductive, physiological and behavioural effects (Longcore and Rich 2004, Navara and Nelson 2007, Perkin et al. 2011, Honnen et al. 2016). Most ecological research has focused on individual species but how ALAN might affect populations or communities is rarely studied (Gaston et al. 2015, Spoelstra et al. 2015). Many urban and sub-urban areas, including residential and industrial areas and roads, are located close to rivers, streams, and lakes because humans have long tended to build settlements close to freshwaters (Kummu et al. 2011). As a result, many freshwater ecosystems are exposed to ALAN. In mountainous areas, many oligotrophic streams can be particularly exposed to ALAN as they are often clear and shallow and the illumination can easily reach the bottom (Moore et al. 2006).

Many aquatic animals, including aquatic macroinvertebrates, use natural diel light/dark cycles to regulate their diel behaviour (Hölker et al. 2010, Perkin et al. 2011, Perkin et al. 2014b). Disruption of natural light/dark cycles by ALAN may therefore alter diel activity patterns in these organisms. In streams, many benthic macroinvertebrates feed on the substrate during the day, but detach and drift at night to minimize the risk of predation by drift-feeding fish (Allan 1978, Brittain and Eickeland 1988). Clear light/dark cycles of drift in many taxa led to early studies of how light can affect this behaviour. It has been observed that nocturnal light level higher than 10^{-3} lux can reduce drift in the stonefly *Phasganophora capitate*, and in the mayflies *Ephemerella* and *Stenoneina*. Intensities between 0.1 and 1 lux are enough to suppress drift in several taxa such as *Baetis* and *Gammarus* (Tanaka 1960, Holt and Waters 1967, Perkin et al. 2014a, Perkin et al. 2014b) likely because of increased risk of predation (Waters 1972, Flecker 1992). Light intensities of 1 lux were also linked to decreased proportions of scrapers (43%) and filterers (83.4%) (Perkin et al. 2014b). If one of the main effect of ALAN on macroinvertebrates is the inhibition of drift behaviour, macroinvertebrate communities might be more sensitive to ALAN when the abundance of drift is highest. In temperate regions, drift undergoes seasonal fluctuations related to abiotic and biotic variables. These include current/discharge, photoperiod, temperature, benthic densities, predators and life cycle stage (Brittain and Eickeland 1988, Shearer et al. 2002, Robinson et al. 2002, Hieber et al. 2003).

The responses of macroinvertebrates to light can differ among species. Most macroinvertebrates in streams show negative phototaxis (Wodsedalek 1911, Moon 1940, Hughes 1966) while some display positive phototaxis, such as *Baetis* and *Simulium* (Hughes 1966, Scherer 1962). ALAN may therefore induce taxa-specific responses in macroinvertebrate communities, although the impact of ALAN on macroinvertebrate community composition and function is still largely unknown.

We used stream-side flumes, fed by a relatively pristine sub-alpine stream, to simulate nocturnal conditions of a stream exposed to ALAN at levels comparable to those found in urban and suburban areas. We measured the effects of ALAN on density and on taxonomic and functional composition of benthic macroinvertebrate communities over a period of four weeks. During the first week, we assessed the effect of ALAN on ALAN-naïve communities. During the third week, we assessed the effect of ALAN on communities that had already been exposed for 2 weeks. After the third week, all flumes were returned to a natural light/dark cycle and the communities were again compared after the fourth week, i.e. after one week without ALAN, to assess community resilience. The experiment was performed in spring and again in autumn to account for seasonal differences in community composition and environmental factors.

2.3 Methods

2.3.1 Study site

The study was conducted using a set of five metal flumes installed on the right bank of the Fersina river, in Trentino Province, north-eastern Italy (see Fig 1a, b). The Fersina is a sub-alpine 2nd order stream (630 a.s.l.) that is 14 km long and part of the 171 km^2 Adige River watershed. The flumes have been used for ecohydrological studies on periphyton (Cashman et al., 2016) and benthic macroinvertebrates (Carolli et al. 2012, Bruno et al. 2013, Bruno et al. 2016). The experimental flumes and the entire upstream section of the stream have never experienced ALAN. The five flumes are 20 m long and 30 cm wide with side walls that are either 30 cm high (flumes A-C) or 50 cm high (flumes D, E) (see Fig. 1d). Flumes are fed with water that is diverted from the Fersina river into a sluice box upstream of the flumes. A metal mesh (3 x 5 cm) prevents large debris and fish from entering the system but allows macroinvertebrates to colonize the flumes. The flumes are filled to the same depth with 20 cm layer of cobbles of approximately 10 cm diameter and a layer of sand/gravel deposited by the water flow. Six months prior to the experiment, water discharge was set by sluice gates to a baseflow of 0.05 $m^3 s^{-1}$ and velocity of 0.4 $m s^{-1}$ in each flume. Each flume was divided into upper and lower sections of 10 m length each (see Fig. 1d).

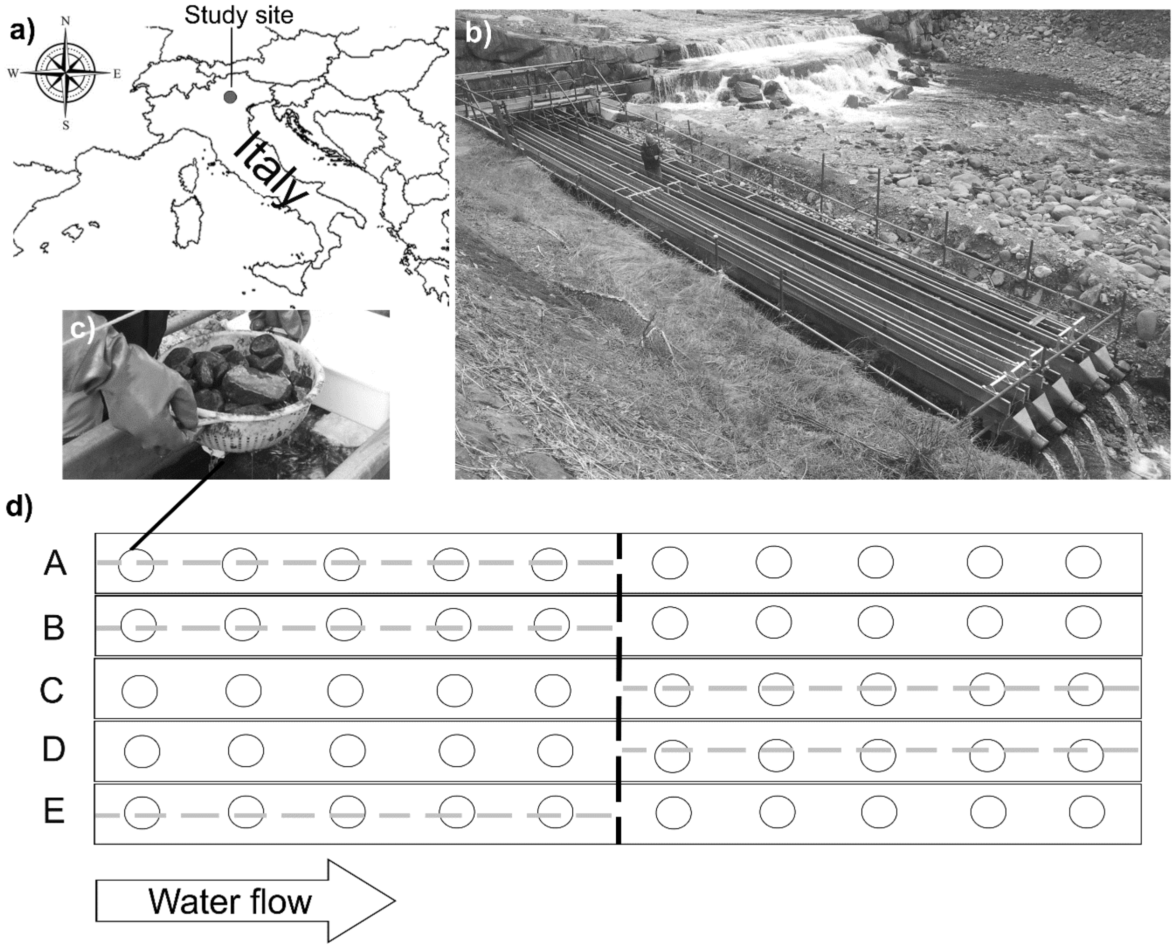


Figure 1. Study site in Trentino, Italy (46° 04′ 32″ N, 11° 16′ 24″ E) (a). View of the set of five streamside experimental flumes on the Fersina river (Trentino, NE Italy). View taken from downstream, flow runs from top to bottom (b). Baskets filled with cobbles and gravel used as artificial substrates to collect macroinvertebrates(c). Position of the baskets in the illuminated (dashed lines) and un-illuminated sections (d).

2.3.2 Animal collection and experimental design

Macroinvertebrates were collected in spring and autumn 2014 using slotted circular baskets (i.e., pasta colanders, 57.4 x 27.4 x 13.2 cm) (Fig. 1c). All baskets were filled with substrate collected from the Fersina river and composed of the same proportion of grain size classes: fine (4-8mm) medium (8-16mm) and coarse (32-64mm) gravel (Fig. 1c). On March 4 (spring) and September 1 (autumn), ten baskets were placed into each flume (Fig. 1d) and left there for 23 days to undergo natural colonization by ALAN-naïve macroinvertebrates. On March 31 and September 24, battery-powered warm-white LED strips (12 V, 3300 K, Barthelme, Nürnberg, Germany, see light spectra in Appendix S1) were installed on wires mounted 30 cm above the water surface. Lights were installed in each flume, above either the upper or lower section (chosen randomly). This resulted in five treatment and five control sections of 10 m each (see Fig. 1d). At night, flume sections were separated by foil curtains to prevent any light from reaching the adjacent control sections. For three weeks, lights were turned on and off at civil twilight and dawn using an automatic timer. Light intensity was measured with an ILT1700 underwater photometer (International Light Technologies Inc., Peabody, Massachusetts, USA). Illumination reaching the bottom of the flumes in the light treatment was 20.3 ± 1.8 lux (mean and SD, $n = 20$; *ca*. 0.31 $\mu mol\ m^{-2}\ s^{-1}$).

A complete scheme of the sample collection design is depicted in Appendix S2. In both spring and autumn, one sample was collected from each flume section prior to starting the illumination treatment. This was done to assess initial community density and composition. After the illumination treatment began, we collected samples once per week for 3 weeks. A fifth collection was conducted 1 week after the artificial illumination was turned off. Between the first and the second sampling (i.e., during the first week) and between the third and the fourth sampling (i.e., during the third week), drift nets (350 µm mesh size) were placed in each flume; one at the sluice gate (water inflow), and one between upper and lower sections. Drifting material was collected during these periods every morning (8 am) and evening (8 pm). Drift nets were used to prevent incoming macroinvertebrate drift, and to isolate control and treatment sections. Between the second and the third sampling (i.e., during the second week) the drift nets were removed to allow incoming macroinvertebrates to recolonize the flumes. Recolonization in the treatment sections occurred under artificial illumination.

Between the fourth and the fifth sampling (i.e., during the fourth week), drift nets were again removed for colonization in absence of artificial illumination (See Appendix S2 for a scheme of the experimental design).

At each sampling occasion, we randomly selected the baskets to be collected (i.e., one from each of the 10 sections). All invertebrates were collected from the baskets and removed from the individual stones and the basket itself into a plastic tray, filtered through a 350-um mesh and transferred to a 70% ethanol solution. In the laboratory, macroinvertebrates were identified to species or genus (e.g. Ephemeroptera, Plecoptera and Trichoptera) and family level (e.g. Chironomidae, Simuliidae; see Appendix S3 for the complete taxonomic list) following Campaioli et al. (1994), Campaioli et al. (1999), Lechthaler and Stockinger (2005) and Fochetti and Ravizza (2009).

Handheld meters (WTW GmbH, Weilheim, Germany) were used to measure oxygen, pH, conductivity and turbidity in each flume. A hand-held current meter (Global Water Flow Probe, Global Water Instrumentation, College Station, Texas, USA) was used to measure flow velocity. Measurements were conducted on a weekly basis (see Appendix S4).

2.3.3 Feeding groups

Macroinvertebrates were classified into one of six feeding categories (deposit feeders, shredders, scrapers, filterers, piercer and predators) following Usseglio-Polatera et al. (2000) and Tachet et al. (2002). Trait information was collected at the genus level for Ephemeroptera, Plecoptera, and Thrichoptera. Family level was used for the remaining taxa. A fuzzy coding approach was used to determine the affinity of each taxon to each category, thus accounting for intra-genus and intra-family variation (Chevenet et al. 1994). Affinity scores ranged between 0 and 3 or 0 and 5, and reflected the relative strength of association of a taxon for a given trait category (Dolédec et al. 2006). Affinity scores were multiplied by the relative abundance of each taxon within each basket. We obtained a traits-by-basket matrix that contained the relative abundance of each feeding group per basket (Larsen and Ormerod 2010, Manfrin et al. 2013, Manfrin et al. 2016).

2.3.4 Data analysis design

We used three statistical analyses, each based on a replicated B.A.C.I. (before-after, control-impact) design (Stewart-Oaten et al. 1986), to assess the effects of ALAN on benthic macroinvertebrate communities in the flume sections. A first analysis on ALAN-naïve communities compared the period before the start of the illumination, when both control and treatment sections were subject to the natural light/dark cycles, with the second period during which treatment sections were lit at night and the control sections were not (See Appendix S2). The second analysis considered communities already exposed to ALAN (ALAN-exposed). After allowing new colonization during the second week, communities in control and treatment sections were compared at the beginning (ALAN-exposed, before) and at the end of the third week (ALAN-exposed, after) (see Appendix S2). A third analysis was performed to assess whether macroinvertebrate communities were able to recover to the undisturbed state after the end of the illumination (i.e. resilience). Communities recolonizing for 1 week in absence of illumination (post-ALAN, end), following the three weeks of artificial illumination, were compared to communities in the control sections and to ALAN-naïve communities prior to illumination (ALAN-naïve, before) (See Appendix S2). For each analysis, macroinvertebrate densities, community taxonomical and feeding group composition were analysed. Analyses were performed separately for data from spring and autumn 2014.

2.3.5 Statistical analysis

For all analyses, the number of animals was standardized per unit of surface area of the substrate in the baskets (i.e. density, ind m^{-2}). The area of each basket was measured as the sum of areas of each stone of which they were composed. Size of pebbles and gravel were assessed using sieves and surface was calculated considering them as spheres. We included in the macroinvertebrate data analysis only final instar larvae (and adults for species with exclusively aquatic life cycles, e.g. *Asellus* sp.).

The effect of ALAN on macroinvertebrate density was examined using linear mixed effects (LME) models as implemented in the “lme4” package (Bates et al. 2007) for R (R Core Team, 2015). A first model (Model I) was used for the BACI analysis of the ALAN-naïve communities. The fixed factors “section” (control, treatment) and “period” (before, after) were included with their interaction (see Appendix S2). A second model (Model II) was used to assess the effect of ALAN on ALAN-exposed communities. Similarly to the analysis of the ALAN-naïve communities, Model II considered the fixed factors “section” and “period” including their interaction (see Appendix S2). A third model (Model III) was used to assess the macroinvertebrate community after the end of the illumination compared to prior to the experiment. Model III considered the fixed factors “section” and “period” (ALAN-naïve, before and post-ALAN, end) (see Appendix S2) including their interaction. For all LME models, fixed factors were tested and compared with a reduced model (i.e. without the fixed factors) using likelihood ratio tests (χ^2) (Pinheiro and Bates 1995). “Flume” was considered as a random factor to account for multiple sampling. The variance explained by the model was calculated as marginal (R^2m) (Nakagawa and Schielzeth 2013) using the MuMln package (Barton 2011) for R. The distribution of residuals was assessed using Wilk-Shapiro tests (Shapiro and Wilk 1965) and qq-plots (Wilk and Gnanadesikan 1968). For each LME with a significant interaction we performed contrast analysis as pairwise comparison using least-squares means (LSM) using the lsmeans package (Lenth 2016) for R. For each Model (I, II, III), comparisons were performed between control and treatment section within the same period and between the same section (i.e. control or treatment) between two different periods. Benjamini-Hochberg corrected α-values (Waite and Campbell 2006) were used to control for inflated false discovery rates.

Compositional differences among baskets were computed as Bray-Curtis dissimilarities (Beals 1984). Prior to the analysis, we standardized the dataset using a chord transformation in order to reduce the dominance of the most common species. Similarly, to the three LME models, two-way perMANOVAs (Adonis function) were calculated for the three comparisons (i.e. ALAN-naïve; ALAN-exposed; post-ALAN). To test for compositional dissimilarity, factors “section” and “period” (see above, Appendix S2) were included with their interactions. “Flumes” was used as blocking factor to account for multiple sampling. Where a significant interaction effect was detected, we performed a one-way perMANOVA as pairwise comparison to test for a compositional difference between control and treatment section within the same period and between the same section (i.e. control or treatment) between two different periods. Similarity percentage (SIMPER) analysis (Clarke 1993) was used to identify a ranked list of taxa that cumulatively contributed more than 70% to the significant (after perMANOVA) difference between control and treatment sections. To visualize differences in taxa composition among sections and conditions we produced non-metric multidimensional scaling (nMDS) plots.

Similarly to the analysis of the taxa composition, we used a two-way perMANOVA and nMDS on feeding group composition and a SIMPER analysis to identify feeding groups that contributed to the significant (after perMANOVA) difference between control and treatment sections. All the compositional analysis were performed using the "Vegan" package (Oksanen et al. 2013) for R. All dissimilarity matrices were tested for homogeneity of multivariate dispersion (Anderson 2006). To control false discovery rate, we used the Benjamini-Hochberg procedure.

2.4 Results

2.4.1 ALAN-naïve communities

LME model I, analysing ALAN-naïve communities, indicated significant variation in macroinvertebrate density (ind m^{-2}) among flume sections (i.e. treatment vs control) and periods (before, after) only in spring (Tab. 1, Fig. 2). After 1 week of illumination macroinvertebrate densities in the control sections decreased by 2- and 3-fold, compared to the treatment section in the same period and to the control sections before the experiment started (t_{19} = 4.85; p <0.001, Fig. 2a). There was no difference in macroinvertebrate density between treatment and control sections prior to ALAN nor between treatment sections prior and after ALAN (Fig. 2a).

Macroinvertebrate taxonomical composition in ALAN-naïve communities differed significantly among sections and periods only in spring ($F_{1,16}$ = 6.94, p<0.001, Fig.3). Control sections differed from treatment sections after 1 week of exposure to ALAN ($F_{1,8}$ = 4.87, p = 0.01, Fig. 3a). SIMPER analysis indicated that Chironomidae (contribution to dissimilarity, CD = 23%) and *Baetis* spp. (CD = 13%) were 4 times more abundant in the treatment sections. In the control sections, taxonomical composition after 1 week of exposure to ALAN also differed from the composition prior to ALAN ($F_{1,8}$ = 11.02, p< 0.001, Fig. 3a). Differences were largely determined by Chironomidae and the stonefly *Brachyptera risi* which were 7 and 24 times (CD = 0.37% and 7%) more abundant in the control sections at the start of the experiment compared to 1 week later. The taxonomical composition in the treatment sections did not change after the week of illumination (Fig.3a).

Feeding group composition in ALAN-naïve communities differed significantly among sections and periods, only in spring ($F_{1,\ 16}$ = 9.28, p=0.007, Fig.4). Control sections differed from treatment sections after 1 week of exposure to ALAN ($F_{1,\ 8}$ = 11.69, p =0.008, Fig. 4a). This difference was due to scrapers being more abundant in the illuminated sections (1.5-fold; CD = 7%) and filterers (e.g. Simuliidae and net-spinning caddisflies of the family Hydropsychidae) being less abundant in the illuminated sections (2-fold; CD = 15%). Feeding group composition also differed in control sections after 1 week of illumination compared to the period prior to ALAN ($F_{1,\ 8}$ = 27.36, p =0.01, Fig. 4a). Specifically, filterers were twice as abundant (contribution to dissimilarity= 17%) and shredders were 4-times more abundant (CD = 17%) in control sections after 1 week of exposure to ALAN compared to prior to the start of the illumination. In the treatment sections, prior and after 1 week of exposure to ALAN, the feeding group composition did not change (see Fig.4a).

2.4.2 ALAN-exposed communities

Analysis of the effect of ALAN on macroinvertebrates already exposed to the light treatment (LME model II) showed significant difference in density between lit and dark sections in spring (Tab. 1, Fig. 2). Densities were 2-fold higher in illuminated sections after 2 weeks (t_{19} = -2.14; p =0.04, Fig. 2a) and also after 3 weeks of exposure to illumination (t_{19} = -2.61; p =0.01, Fig. 2a). No difference in either taxonomic or functional composition was detected among sections and periods in either spring or autumn (Fig. 3, Fig. 4)

2.4.3 Community resilience post-ALAN

LME model III indicated a significant difference among sections and periods (see Appendix S2) only in spring (Table 1, Fig. 2). Density was persistently two times higher in the previously-illuminated sections compared to the control sections in the same period (t_{19} = -2.72; p =0.01, Fig. 2a). At the same time, density in the control sections after the end of the experiment were 2 times lower than in the control sections prior to the start of the illumination (t_{19} = 2.55; p =0.02, Fig. 2a). perMANOVA analyses did not identify difference in community composition among sections and periods. However, composition differed between the period prior to the start and after the end of the experiment for both spring ($F_{1, 16}$ = 14.61; p=0.002) and autumn ($F_{1, 16}$ = 6.06; p=0.03) (see Fig. 3c). Difference in feeding group composition was found between the period prior to the start and after the end of the experiment in spring ($F_{1, 16}$= 5.91; p=0.003) but not in autumn (see Fig. 4).

Table 1. Comparison of macroinvertebrate density (ind m^{-2}) between control and treatment (lit) flume sections for communities exposed to ALAN for 1 week (ALAN-naïve); communities exposed to ALAN for 2 weeks compared to communities exposed for 3 weeks (ALAN-exposed); communities 1 week after the end of the illumination compared with the ALAN-naïve communities (post-ALAN). LME likelihood ratio test (χ^2) and independent variable significance *F*-test are shown. Asterisks indicate significant effects (*** = p<0.001; ** = p<0.01; * = p<0.05).

Season	Analysis	χ^2	Model factors	*F*- statistic
Spring	ALAN-naïve	21.29***	Period	$F_{1,15}$=27.37***
			Treatment	$F_{1,15}$=6.09*
			Period x Treatment	$F_{1,15}$=5.99*
	ALAN-exposed	10.93*	Period	NS
			Treatment	$F_{1,20}$=14.13**
			Period x Treatment	NS
	post-ALAN	9.88*	Period	NS
			Treatment	$F_{1,20}$=4.65*
			Period x Treatment	$F_{1,20}$=4.58*
Autumn	ALAN-naïve	NS		
	ALAN-exposed	17.85***	Period	$F_{1,15}$=23.64***
			Treatment	NS
			Period x Treatment	NS
	post-ALAN	NS		

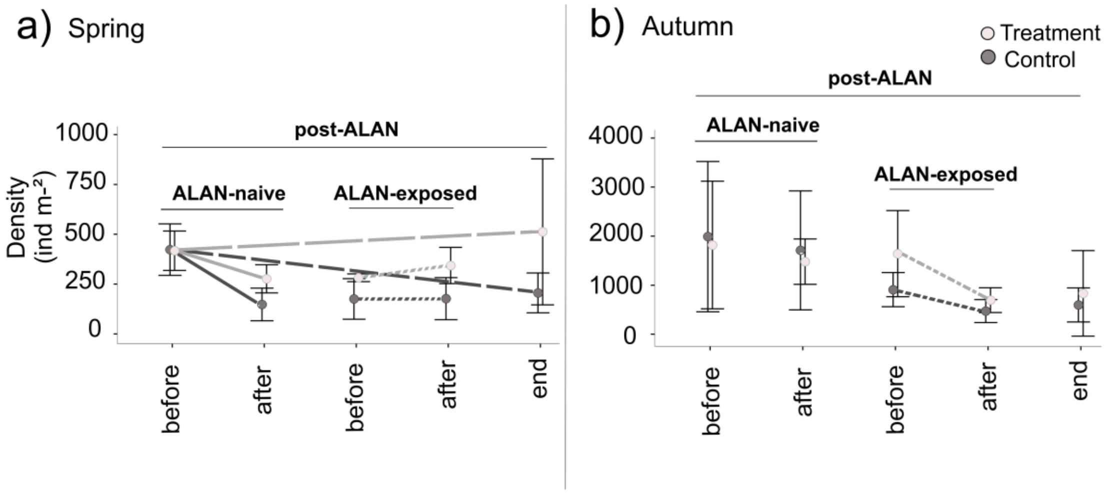


Figure 2. Comparison of macroinvertebrate density (ind m^{-2}) between control (dark grey dashed lines and dots) and treatment sections (light grey dotted lines and dots) for spring (a) and autumn (b) experiments, to assess the effect of ALAN on: ALAN-naïve communities exposed for 1 week (ALAN-naïve); communities exposed to ALAN for 2 weeks compared to communities exposed for 3 weeks (ALAN-exposed); communities 1 week after the end of the illumination and ALAN-naïve communities prior to the start of the illumination (post-ALAN) (See Appendix S2). Significant comparisons (LME) are shown for each analysis as solid lines (ALAN-naïve); dotted lines (ALAN-exposed); dashed lines (post-ALAN).

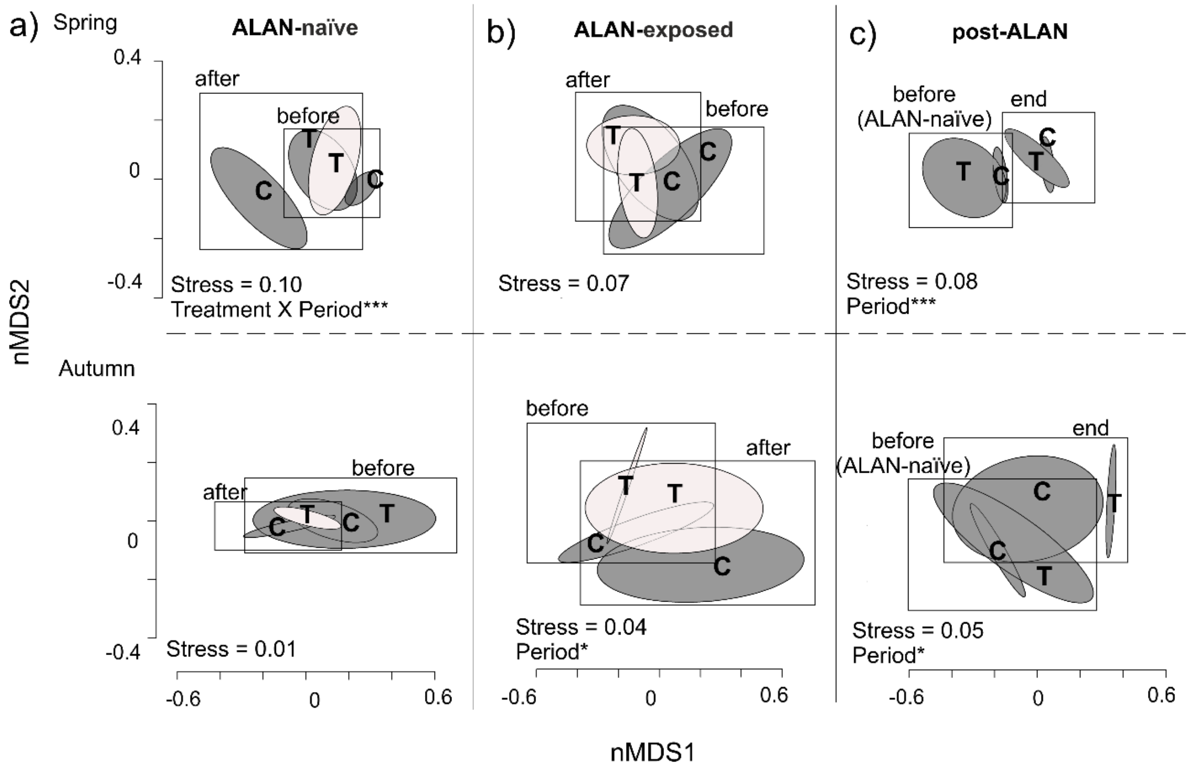


Figure 3. Community composition of macroinvertebrates is illustrated using non-metric multidimensional scaling (nMDS). Effect of ALAN on composition is assessed between the control (C) and the treatment (T) sections on: ALAN-naïve communities exposed for 1 week (ALAN-naïve) (a); communities exposed to ALAN for 2 weeks compared to communities exposed for 3 weeks (ALAN-exposed) (b); communities 1 week after the end of the illumination compared to ALAN-naïve communities (post-ALAN) (c). Ellipses represent 95% confidence areas of treatment and control sections. Significant perMANOVA factors are shown. Asterisks indicate significance (*** = p<0.001; ** = p<0.01; * = p<0.05). Analyses were performed for spring and autumn samples.

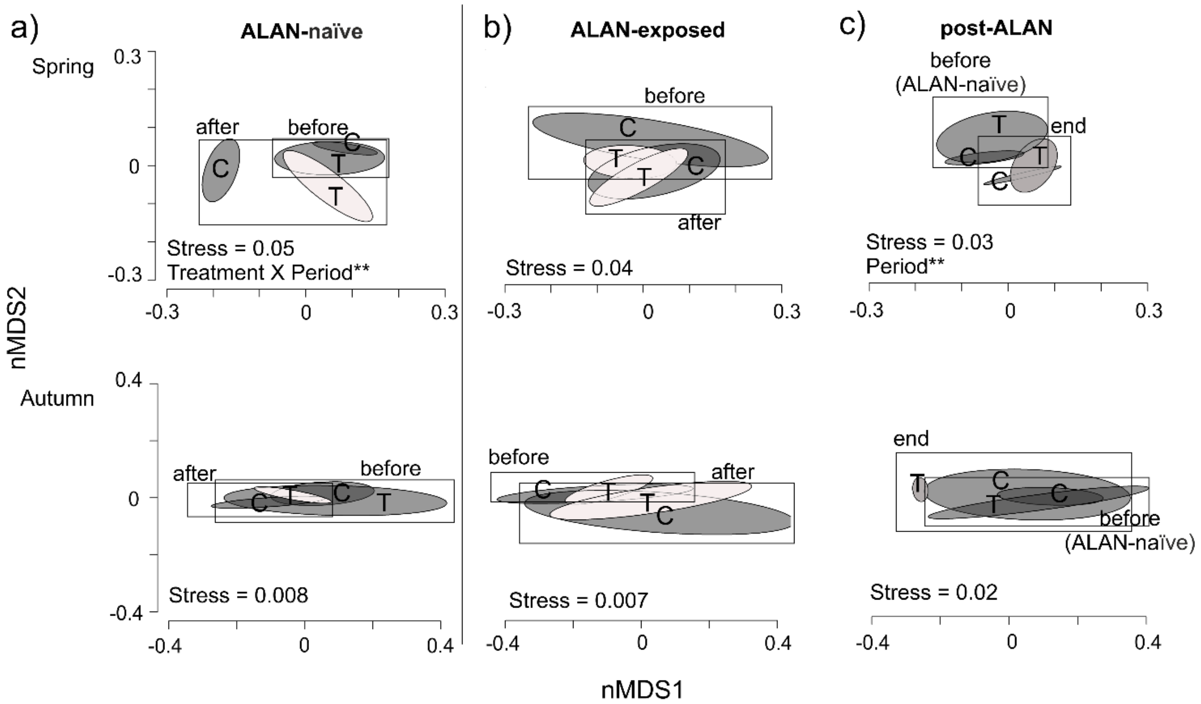


Figure 4. Macroinvertebrate feeding group composition is illustrated using non-metric multidimensional scaling (nMDS). Effect of ALAN on composition is assessed between the control (C) and the treatment (T) sections on: ALAN-naïve communities exposed for 1 week (ALAN-naïve) (a); communities exposed to ALAN for 2 weeks compared to communities exposed for 3 weeks (ALAN-exposed) (b); communities 1 week after the end of the illumination compared to ALAN-naïve communities (post-ALAN) (c). Ellipses represent 95% confidence areas of treatment and control sections. Significant perMANOVA factors are shown. Asterisks indicate significance (*** = <0.001; ** = <0.01; * = <0.05). Analyses were performed for spring and autumn samples.

2.5 Discussion

2.5.1 ALAN-naïve communities

We observed changes in macroinvertebrate densities in spring, when densities remained significantly higher in illuminated sections after one week of exposure to ALAN, suggesting that drift was inhibited. In the control sections, there was a decrease in macroinvertebrate density, indicating that individuals drifted out. After the initial macroinvertebrate colonization from the Fersina river, drift-nets were installed at the upstream end of each section to prevent further colonization (see Appendix S2). As a result, observed effects were assumed to result from differences in out-going drift.

Most stream invertebrates are known to actively enter the water column (i.e., behavioural drift) to escape predators, to avoid extreme conditions, or to search for patchily distributed food resources (Brittain and Eikeland 1988, Rader 1997). The trade-off between maximizing energy intake from new food patches and minimizing mortality by predation while drifting usually results in nocturnal peaks in drift (see Naman et al. 2016 and references therein). Photoperiod therefore plays a key role in regulating drift dynamics (Brittain and Eikeland 1988). It is generally understood that changes in drifting behaviour are not controlled by endogenous circadian patterns (Bishop 1969). Low light intensities (between 10^{-2} and 10^{-3} lux) have been found to decrease macroinvertebrate drift (Bishop 1969). Perkin et al. (2014b) recorded a 50% reduction of night-time drift of aquatic invertebrates in lit reaches compared to natural (dark) reaches in small forest streams. Similar results are found in our experiment. Reduction of macroinvertebrate density in the control sections due to predation by fish can be excluded since fish were absent from the flumes.

Our results suggest that artificial illumination can induce changes in ALAN-naïve macroinvertebrate communities affecting taxonomical composition of macroinvertebrate communities. Overall density patterns were driven by changes in densities of *Baetis* spp. and of Chironomidae, presumably through reduced drift in illuminated sections. *Baetis* spp. are phototactic (Hughes 1966, Scherer 1962). Exposure to ALAN seems to elicit a firmer attachment to the substrate and a suppression of the animal activity (Hughes 1966, Bishop 1969) resulting in fewer animals entering the drift once exposed to artificial illumination (Anderson 1966, Bishop 1969, Perkin et al. 2014b). Our study confirmed this trend for *Baetis* spp. and Chironomidae which can hide in interstitial crevices beneath stones or in the sand when they perceive increased predation risk (McIntosh and Peckarsky 1999, Hölker and Stief 2005). Although our experimental flumes were devoid of predatory fish, the water comes directly from the adjacent Fersina river and thus fish chemical cues were still perceived by benthic macroinvertebrate (McIntosh et al. 1999).

Food intake while hiding can be significantly lower than when feeding on exposed stone surfaces (Culp et al. 1991) and the energy costs of this latency period can have important consequences for larval fitness (Power 1984, Peckarsky and McIntosh 1998). It is unlikely that higher abundance of *Baetis* spp. in the lit sections was the result of higher food availability because the biomass of primary producers in lit sections was lower than in the control sections (Personal observation Maja

Grubisic). Filterers (i.e. Simuliidae, Hydropsychidae) were less abundant under ALAN. Preliminary data confirmed increased drift of Simuliidae and Hydropsychidae at night when animals were exposed to ALAN (data not shown). This may be due to increased intentional drift to avoid predation (Rader 1997). In fact, animals attached to the substrate (e.g. Simuliidae, Hydropsychidae), become easily visible under illumination. For these sessile taxa, unlike *Baetis* spp. and Chironomidae (see above), increased perceived predation risk due to ALAN might result in increased drift as predator avoidance behaviour.

2.5.2 ALAN-exposed communities and community resilience post-ALAN

The effects we observed after one-week exposure to ALAN on macroinvertebrate densities, community composition and feeding groups (see above) persisted into the second and third weeks of ALAN exposure. Prolonged exposure to ALAN did not appear to have an increasing effect on communities, but rather maintained the initial impact.

Communities that were exposed to ALAN for three weeks remained significantly different from control sections even after a return to natural light/dark cycles for one week (see Fig. 2a). One week after the end of the illumination, communities previously exposed to ALAN still contained more animals than the control. This was despite the flumes being equally open to recolonization in this phase of the experiment. No difference in community composition and feeding groups remained between the illuminated and the dark sections, indicating that community structure was able to recover from the impact of ALAN, despite the lower densities in control sections. At the beginning of the second experiment in autumn, approximately 6 months after the end of the first experiment in spring, treatment and control flume sections did not differ in abundance. This indicates that the communities had entirely recovered in the intervening period through recolonization via incoming drift and/or egg deposition by the more mobile adult insects in the summer period.

2.5.3 Seasonality

We observed effects of ALAN on density and composition in ALAN-naïve and pre-exposed macroinvertebrate communities only in spring, while in autumn there was no significant difference between illuminated and dark sections. This may be related to differences in natural drifting patterns that we observed in the two seasons. In spring, after an initial peak of incoming drift measured the night before the start of the illumination (20 g of wet biomass per flume/night), drift decreased substantially (1 g of wet biomass per flume/night) (see Appendix S5). In autumn, overall natural drift levels were higher than in spring across all the experiment (5-15 g of wet biomass per flume/night) (see Appendix S5). In low-altitude temperate streams, pronounced seasonal drift patterns occur due to phenology (e.g. prepupation, emergence) and fluctuations in environmental conditions (Poff DeCino and Ward 1991, Robinson et al. 2002). In our study, spring and autumn communities differed in composition (perMANOVA significance <0.001; results not shown). *Baetis* is often abundant in drift (Water 1972) and was more abundant (20-fold) in autumn than in spring. Chironomidae, which are less prone to drift (Waters 1972), were more abundant (2.5-fold) in spring. The drift of *Baetis* was strongly inhibited by ALAN, therefore it could be expected that the effect of ALAN would be stronger in autumn than in spring. However, an inhibitory effect of ALAN was not observed in autumn.

The higher (4-fold) density of organisms collected across all the autumnal sampling in the benthic substrates compared to spring (see Fig. 2) might also explain the absence of any effect of ALAN in autumn (McIntosh and Peckarsky 1999). Competition for resources or space might have overridden the effect of ALAN by stimulating (or maintaining) drift regardless of ALAN. Naman et al. (2016) underline that when interpreting drift–benthos relationships at small scales (e.g., within a riffle, which is comparable as extension to our experimental flume setting), density dependence does occur and likely reflects within-patch aggregation dynamics such as resource competition. In this context, drift is regulated by density-dependent interactions (Waters 1972, Ciborowski 1983, Allan 1987). When the number of animals exceeds the habitat carrying capacity, high competition for resources (e.g. space and food) stimulates benthic activity and detachment from the substrate (Waters 1972, Corkum 1978, Wiley and Kohler 1981, Hershey et al. 1993).

Macroinvertebrates might vary in sensitivity to ALAN, depending on the seasonal composition of larval developmental stages during the exposure to illumination. Early larval stages of *Baetis* spp*.* have been reported to drift more than larger *Baetis* spp. due to different swimming/sinking abilities (Bruno et al. 2016, and reference therein). Although, we did not investigate the effect of ALAN on different larval instars directly, in our study, early (i.e. first or second) larval instar individuals of *Baetis* spp. were particularly abundant in autumn (A.M. personal observation) when we observed higher drift rate and no evident effect of ALAN on the macroinvertebrate communities.

2.5.4 Ecological implications of ALAN in freshwater ecosystems

Long-term exposure to ALAN, i.e., over several years as is typically the case for outdoor lighting alongside waterways, might result in an even stronger impact than the short-term effects we observed in this study. This may occur through shifts in competitive ability or indirect effect on food-web regulatory processes within and across stream boundaries. The effect of ALAN could also extend to adjacent terrestrial ecosystems. Aquatic insects can be important resource subsidies for consumers in receiving riparian habitats and can contribute to the overall cross-ecosystem food-web stability (Nakano and Murakami 2001, Takimoto et al. 2002, Paetzold et al. 2011). Reduced drift due to ALAN could lead to increased densities in illuminated areas, resulting in increased insect emergence (see Chapter 3). At the same time, increased local macroinvertebrate abundance may lead to increased competition. This might compensate or override the inhibitive effect of ALAN on drift resulting in a density-induced drift that would reduce insect emergence. In both cases ALAN potentially can alter the fluxes of aquatic insects into the terrestrial area, affecting feeding dynamics between aquatic and terrestrial ecosystems. Finding the threshold of density at which the effect of ALAN is overridden by the effect of the density may be of particular interest to better understand drifting dynamics in artificially illuminated freshwater ecosystems.

The results of our study can provide a basis for predicting benthic invertebrate responses to ALAN in streams and rivers in urbanized areas, and should be further integrated and developed in assessing the top-down or bottom-up effects of ALAN on aquatic food webs. Moreover, if most of the benthic invertebrates that are known to respond to alteration of river quality also respond to ALAN, the effects of the latter should not be neglected when conducting impact assessment.

2.6 Acknowledgements

This research was partially carried out within the Erasmus Mundus Joint Doctorate Program SMART (http://www.riverscience.eu) funded by the Education, Audiovisual and Culture Executive Agency (EACEA) of the European Commission. The authors wish to thank the Servizio Bacini Montani, Autonomous Province of Trento, for building and maintaining the flumes, and Lorenzo Forti, Martino Salvaro and Stefan Heller for their help in setting up and conducting the experiments. We thank Bruno Maiolini for his help during the sample collection and in the laboratory analysis, Viktor Baranov and Valentyna Inshina for help during the invertebrate identification, and Francesca Pilotto, Thomas Mehner and Roy van Grunsven for advice on statistical analysis. Funding was also provided by the Federal Ministry of Research and Technology, Germany (BMBF-033L038A) and the Federal Agency for Nature Conservation, Germany (FKZ 3514821700).

2.7 References

Allan, J. D. 1987. Macroinvertebrate drift in a Rocky Mountain stream. Hydrobiologia, **144**: 261-268.

Anderson, N. 1966. Depressant effect of moonlight on activity of aquatic insects. Nature **209**: 319-320.

Anderson, M. J. 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics **62**: 245-253.

Barton, K. 2011. MuMIn: Multi-model inference. R package version 1.0. 0. Vienna, Austria: R Foundation for Statistical Computing. See http://CRAN. R-project. org/package= MuMIn.

Bates, D., D. Sarkar, M. D. Bates, and L. Matrix. 2007. The lme4 package. R package version 2: 1-74.

Beals, E. W. 1984. Bray-Curtis ordination: an effective strategy for analysis of multivariate ecological data. Advances in Ecological Research **14**: 1-55.

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

Brittain, J. E., and T. J. Eikeland 1988. Invertebrate drift—a review. Hydrobiologia **166**: 77-93.

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.

Campaioli, S., P. F. Ghetti, A. Minelli, and S. Ruffo. 1994. Manuale per il riconoscimento dei macroinvertebrati delle acque dolci italiane, Vol. I. Provincia Autonoma di Trento.

Campaioli, S.,P. F. Ghetti, A. Minelli, and S. Ruffo 1999. Manuale per il riconoscimento dei macroinvertebrati delle acque dolci italiane, Vol. II. Provincia Autonoma di Trento.

Carolli, M., M. C. Bruno, A. Siviglia, and B. Maiolini 2012. Responses of benthic invertebrates to abrupt changes of temperature in flume simulations. River Research and Applications **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. Aquatic Sciences **79**: 1-13.

Chevenet, F., S. Dolédec, and D. Chessel. 1994. A fuzzy coding approach for the analysis of long-term ecological data. Freshwater Biology **31**: 295-309.

Ciborowski, J. J.1983. Influence of current velocity, density, and detritus on drift of two mayfly species (Ephemeroptera). Canadian Journal of Zoology **61**: 119-125.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology **18**: 117-143.

Corkum, L. D. 1978. The influence of density and behavioural type on the active entry of two mayfly species (Ephemeroptera) into the water column. Canadian Journal of Zoology **56**: 1201-1206.

Culp, J. M., N. E. Glozier, and G. J. Scrimgeour 1991. Reduction of predation risk under the cover of darkness: avoidance responses of mayfly larvae to a benthic fish. Oecologia **86**: 163-169.

Dolédec, S., N. Phillips, M. Scarsbrook, R. H. Riley, and C. R. Townsend. 2006. Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. Journal of the North American Benthological Society **25**: 44-60.

Flecker, A. S. 1992. Fish predation and the evolution of invertebrate drift periodicity: evidence from neotropical streams. Ecology **73**: 438-448.

Fochetti, R., C. A: Ravizza, and J. M. Tierno De Figueroa. 2009. Plecoptera. Vol 43. Fauna d'Italia. Calderini, Bologna.

Gaston, K. J., M. E. Visser, and F. Hölker. 2015. The biological impacts of artificial light at night: the research challenge. Philosophical Transactions of the Royal Society of London B **370**, 20140133.

Hershey, A. E., J. Pastor, B. J. Peterson, and G. W. Kling. 1993. Stable isotopes resolve the drift paradox for *Baetis* mayflies in an arctic river. Ecology **74**: 2315-2325.

Hieber, M., C. T. Robinson, and U. Uehlinger. 2003. Seasonal and diel patterns of invertebrate drift in different alpine stream types. Freshwater Biology **48**: 1078-1092.

Hölker, F., and P. Stief. 2005. Adaptive behaviour of chironomid larvae (*Chironomus riparius*) in response to chemical stimuli from predators and resource density. Behavioral Ecology and Sociobiology **58**: 256-263.

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

Holt, C.S., and T. F. Waters. 1967. Effect of light intensity on the drift of stream invertebrates. Ecology **48**: 225-234.

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**: 1-22.

Hughes, D. 1966. On the dorsal light response in a mayfly nymph. Animal Behaviour **14:** 13-16.

Kummu, M., H. De 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**: 1-13.

Larsen, S. and S. J. Ormerod. 2010. Combined effects of habitat modification on trait composition and species nestedness in river invertebrates. Biological Conservation **143:** 2638-2646.

Lechthaler, W., and W. Stockinger. 2005. Trichoptera - key to larvae from Central Europe. CD-Rom-Edition, Eutaxa, Vienna.

Lenth, R. V. 2016. Least-squares means: the R Package lsmeans. Journal of Statistical Software **69**: 1-33.

Longcore, T., and C. Rich. 2004. Ecological light pollution. Frontiers in Ecology and the Environment **2**: 191-198.

Manfrin, A., S. Larsen,L. Traversetti,G. Pace, and M. Scalici. 2013. Longitudinal variation of macroinvertebrate communities in a Mediterranean river subjected to multiple anthropogenic stressors. International Review of Hydrobiology **98:** 155-164.

Manfrin, A., L. Traversetti, F. Pilotto,S. Larsen and M. Scalici. 2016. Effect of spatial scale on macroinvertebrate assemblages along a Mediterranean river. Hydrobiologia **765:** 185-196.

McIntosh, A. R., and B. L Peckarsky. 1999. Criteria determining behavioural responses to multiple predators by a stream mayfly. Oikos **85**: 554-564.

McIntosh, A. R., B. L. Peckarsky, and B. W. Taylor. 1999. Rapid size-specific changes in the drift of *Baetis bicaudatus* (Ephemeroptera) caused by alterations in fish odour concentration. Oecologia **118**: 256-264.

Moon, H. 1940. An investigation of the movements of fresh-water invertebrate faunas. The Journal of Animal Ecology **9**: 76-83.

Moore, M.V., S. J Kohler S.J. and Cheers M.S. (2006) Artificial light at night in freshwater habitats and its potential ecological effects. In: Ecological Consequences of Artificial Night Lighting (Eds C. Rich and T. Longcore), pp. 365–384. Island Press, Washington, DC.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution **4**: 133-142.

Nakano, S., and M. Murakami. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Sciences **98**: 166-170.

Naman, S. M., J. S. Rosenfeld,and J. S. Richardson. 2016. Causes and consequences of invertebrate drift in running waters: from individuals to populations and trophic fluxes. Canadian Journal of Fisheries and Aquatic Sciences **73**: 1292-1305.

Narisada, K., and D. Schreuder. 2004. Light pollution and astronomy. In: Light Pollution Handbook pp. 115-138. Springer.

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

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2013. Package ‘vegan’. Community ecology package, version 2. See https://cran.r-project.org/web/packages/vegan/ vegan.pdf.

Paetzold, A., S. Marian, H. W. Philip, and L. Maltby. 2011. Environmental impact propagated by cross-system subsidy: Chronic stream pollution controls riparian spider populations. Ecology **92**: 1711-1716.

Peckarsky, B. L., and A. R. Mcintosh. 1998. Fitness and community consequences of avoiding multiple predators. Oecologia **113**: 565-576.

Perkin, E. K., F. Hölker, S. Heller, and R. Berghahn. 2014a. Artificial light and nocturnal activity in gammarids. PeerJ, e279.

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, K. Tockner, and J. S. Richardson. 2014b. Artificial light as a disturbance to light-naïve streams. Freshwater Biology **59**: 2235-2244.

Pinheiro, J. C., and D. M. Bates. 1995. Approximations to the log-likelihood function in the nonlinear mixed-effects model. Journal of Computational and Graphical Statistics **4**: 12-35.

Poff, N. L., R. D. DeCino, and J. V. Ward. 1991. Size-dependent drift responses of mayflies to experimental hydrologic variation: active predator avoidance or passive hydrodynamic displacement? Oecologia **88**: 577-586.

Power, M. E. 1984. Depth Distributions of Armored Catfish: Predator-Induced Resource Avoidance? Ecology **65**: 523-528.

R Core Team 2015. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2013. Document freely available on the internet at: http://www. r-project. org.

Rader, R. B. 1997. A functional classification of the drift: traits that influence invertebrate availability to salmonids. Canadian Journal of Fisheries and Aquatic Sciences **54**: 1211-1234.

Robinson, C., K. Tockner, and P. Burgherr. 2002. Seasonal patterns in macroinvertebrate drift and seston transport in streams of an alpine glacial flood plain. Freshwater Biology **47**: 985-993.

Scherer, E. 1962. Phototaktisches Verhalten von Fliesswasser-Insektenlarven. Naturwissenschaften **49**: 477-478.

Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality (complete samples). Biometrika **52**: 591-611.

Shearer, K. A., J. W. Hayes, and J. D. Stark. 2002. Temporal and spatial quantification of aquatic invertebrate drift in the Maruia River, South Island, New Zealand. New Zealand Journal of Marine and Freshwater Research. **36**: 529–536.

Spoelstra, K., R. H. A. van Grunsven, M. Donners, P. Gienapp, M. E. Huigens, R. Slaterus, F. Berendse, M. E. Visser, and E. Veenendaal. 2015. Experimental illumination of natural habitat—an experimental set-up to assess the direct and indirect ecological consequences of artificial light of different spectral composition. Philosophical Transactions of the Royal Society of London B: Biological Sciences **370**, 20140129.

Stewart-Oaten, A., W. W. Murdoch, and K. R. Parker. 1986. Environmental impact assessment:" Pseudoreplication" in time? Ecology **67**: 929-940.

Tachet, H., P. Richoux, M. Bournaud, and P. Usseglio-Polatera. 2002. Invertébrés d'Eau Douce (2nd corrected impression). CNRS éditions, Paris.

Takimoto, G., T. Iwata, and M. Murakami. 2002. Seasonal subsidy stabilizes food web dynamics: balance in a heterogeneous landscape. Ecological Research **17**: 433-439.

Tanaka, H. 1960. On the daily change of the drifting of benthic animals in stream, especially on the types of daily change observed in taxonomic groups of insects. Bulletin of Freshwater Fisheries Research Laboratory **9**: 13-24.

Usseglio-Polatera, P.,M. Bournaud, P. Richoux,and H. Tachet. 2000. Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. Freshwater Biology **43**: 175-205.

Waite, T. A., and L. G. Campbell. 2006. Controlling the false discovery rate and increasing statistical power in ecological studies. Ecoscience **13**: 439-442.

Waters, T. F. 1972. The drift of stream insects. Annual Review of Entomology **17**: 253-272.

Wiley, M. J., and S. L. Kohler. 1981. An assessment of biological interactions in an epilithic stream community using time-lapse cinematography. Hydrobiologia **78**: 183-188.

Wilk, M. B., and R. Gnanadesikan. 1968. Probability plotting methods for the analysis for the analysis of data. Biometrika **55**: 1-17.

Wodsedalek, J. E. 1911. Phototactic reactions and their reversal in the may-fly nymphs *Heptagenia interpunctata* (Say). The Biological Bulletin **21**: 265-271.

3. Artificial light at night alters flux across ecosystem boundaries and community structure in the recipient ecosystem

Manfrin A.[1, 2, 3], Larsen S.[4], Weiß N.[1, 2], van Grunsven R. H. A.[1], Weiß N-S.[1, 2], Wohlfahrt S.[1, 2], Singer G.[1], Monaghan M. T.[1*], Hölker F.[1*]

[1] *Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

[2]*Department of Biology-Chemistry-Pharmacy, Freie Universität Berlin, Berlin, Germany*

[3]*School of Geography, Queen Mary University of London, London, England, UK*

[4] *German Centre for Integrative Biodiversity Research, Leipzig, Germany*

* Authors contributed equally

3.1 Abstract

Artificial light at night (ALAN) is a widespread alteration of the natural environment that can disrupt animal movement and activity patterns. These changes in movement and activity may have the capacity to affect the functioning of ecosystems. Many freshwater animals move across ecosystem boundaries into the adjacent riparian and terrestrial surroundings as part of their life cycle and constitute important trophic subsidies for riparian consumers. ALAN can change the movement patterns of emergent freshwater insects, with potential implications for adjacent riparian areas.

We report results from a two-year field experiment set up in an ALAN-naïve environment to quantify ALAN-impacts on the freshwater-terrestrial linkage. Using newly erected streetlights we experimentally illuminated an agricultural drainage ditch and evaluated changes in the abundance and community composition of emerging aquatic (emergence traps), flying (air eclector traps), and ground-dwelling (pitfall traps) arthropods. These were compared with a non-illuminated yet environmentally similar nearby system within and between years using general linear models.

Aquatic insect emergence was 3-fold higher in ALAN-exposed traps and aquatic insects comprised 85% of flying insect abundance compared to 50% in the unlit controls. The abundance of flying aquatic insects caught in ALAN-exposed traps was up to 460-fold higher than in control traps. The abundance of flying terrestrial insects was up to 68-fold higher. There was an increased abundance of night-active predators (*Pachygnatha clercki*, Opiliones) and a decreased abundance of nocturnal ground beetles (*Agonum duftschmidi*, *Pterostichus nigrita*, *Carabus granulatus*) in ALAN-exposed traps. Several night-active taxa extended their activity into the day when exposed to ALAN.

Our results indicate that ALAN can have a dramatic effect on aquatic insect emergence and on the community composition of riparian predators and scavengers. We conclude that these effects were linked and that the increased aquatic to terrestrial subsidy flux cascaded through the riparian food web. Our work provides strong evidence for ALAN effects on fundamental processes that link ecosystems at the organism, community, and ecosystem scales. Given the large number of streetlights present along freshwater bodies, we argue that adequate conservation measures require consideration of the effects of artificial illumination.

3.2 Introduction

The recent global increase in the use of artificial light at night (ALAN) and the associated effects on wild organisms have received considerable attention in the last few years (Hölker et al. 2010). It has become clear that ALAN can have multiple effects on both terrestrial and aquatic organisms, exerting reproductive, physiological and behavioural effects (Longcore and Rich 2004, Navara and Nelson 2007, Perkin et al. 2011, Kurvers and Hölker 2015, Honnen et al. 2016). In freshwaters, ALAN can disrupt natural behavioural patterns such as diel vertical migration of zooplankton and arthropod drift (Bishop 1969, Moore et al. 2001, Perkin et al. 2014b) and can alter fish predatory efficiency (Tabor et al. 2004). In terrestrial habitats, ALAN can affect arthropod dispersal patterns (Eisenbeis et al. 2006, Degen et al. 2016) and reproductive behaviour (van Geffen et al. 2014). Most research has focused on appraising the direct effects of ALAN on individual species, and few studies have considered effects on whole communities (Hölker et al. 2015, Holzhauer et al. 2015, Spoelstra et al. 2015). The extent to which ALAN affects the functioning of ecosystems is poorly understood to date (Gaston et al. 2015). One of the mechanisms through which ALAN can exert ecological effects is by changing movement and dispersal patterns of organisms, eventually altering their distribution within and across ecosystems with under-explored consequences.

Artificial illumination is particularly widespread near water bodies where human populations are concentrated (Kummu et al. 2011, Perkin et al. 2011), yet our understanding of the effects on freshwater ecosystems and adjacent terrestrial ecosystems remains limited. These ecosystems are connected by important fluxes of energy and matter. In freshwater ecosystems, terrestrially derived carbon and nutrients support aquatic metabolism (Wallace et al. 1999, Mehner et al. 2005), and the emergence of adult aquatic insects, amphibians and fish can provide subsidies for a wide range of terrestrial consumers (Marczak and Richardson 2007, Bartels et al. 2012). Subsidy fluxes can be reciprocal, benefitting consumers in both habitats at different times (Polis et al. 1997, Nakano and Murakami 2001, Richardson et al. 2010) and they can contribute to overall food-web and ecosystem stability (Takimoto et al. 2002). Previous studies have found that ALAN can attract aquatic insects to terrestrial ecosystems, disrupt natural inland dispersal patterns and increase mortality by exhaustion and predation (Horváth et al. 2009, Perkin et al. 2014a). These studies raise the possibility that ALAN can change the magnitude and dynamics of subsidies between aquatic and terrestrial ecosystems (Perkin et al. 2011, Meyer and Sullivan 2013).

Here we investigate the impact of ALAN on the aquatic-terrestrial linkage of a lowland stream and its adjacent terrestrial ecosystem by assessing aquatic and terrestrial arthropod communities. Many published related studies fail to disentangle ALAN from confounding factors, as they rely on simplistic comparisons between (pre-existing) illuminated and dark areas, thus providing only limited insight into ALAN-specific ecological effects. Artificial illumination is confounded with multiple anthropogenic stressors known to strongly drive ecosystem change, such as urbanization, sealing (paving) of the ground, increased noise, and chemical pollution (Perkin et al. 2011). We report results from a large-scale field experiment in which we introduced commercial streetlights to a previously ALAN-naïve area in a controlled manner. Streetlights were installed along a drainage ditch and in the adjacent riparian areas at two sites: one site was illuminated at night and the other remained dark to serve as a control. This experimental setup controls for other aspects of urbanisation and the use of unlit streetlights at the control site excludes confounding effects of the physical structure that lights provide. We investigated the effect of illumination on (i) aquatic insect emergence, considered as a primary source of aquatic subsidies to the terrestrial system; (ii) the spatial and temporal distribution of flying aquatic and terrestrial insects in the riparian environment; and (iii) the abundance and composition of riparian ground-dwelling predator and scavenger communities.

3.3 Methods

3.3.1 Study area

The field experiment was carried out using a large-scale experimental infrastructure fully described by Holzhauer et al. (2015). It is located in the Westhavelland Nature Park and within a 750-km² International Dark-Sky Reserve that is one of the least illuminated areas in Germany (International Dark Sky Association, IDA 2015). The area is characterized by an extensive system of agricultural drainage ditches (Fig. 1a, b). In April 2012, two identically managed grassland areas with no prior exposure to ALAN were selected for a long-term experiment to study ecological impacts of ALAN.

The two sites are separated by a distance of ca. 600 m (ca. 800 m along the drainage ditch) and a row of trees. Both sites were equipped with 3 parallel rows (3 m, 23 m, and 43 m away from the water) of 4 conventional 4.75 m high streetlights located 20 m apart (Fig. 1c) and with one 70-W high-pressure sodium lamp each (OSRAM VIALOX NAV-T Super 4Y). Ecological monitoring started at the beginning of May 2012, prior to any illumination. From July 25 onward, one site (the treatment) was illuminated at night, i.e., one set of streetlights was switched on between civil twilight at dusk and dawn. The control (dark) site remained dark, yet provided identical physical structure (see Holzhauer et al. 2015 fur further details).

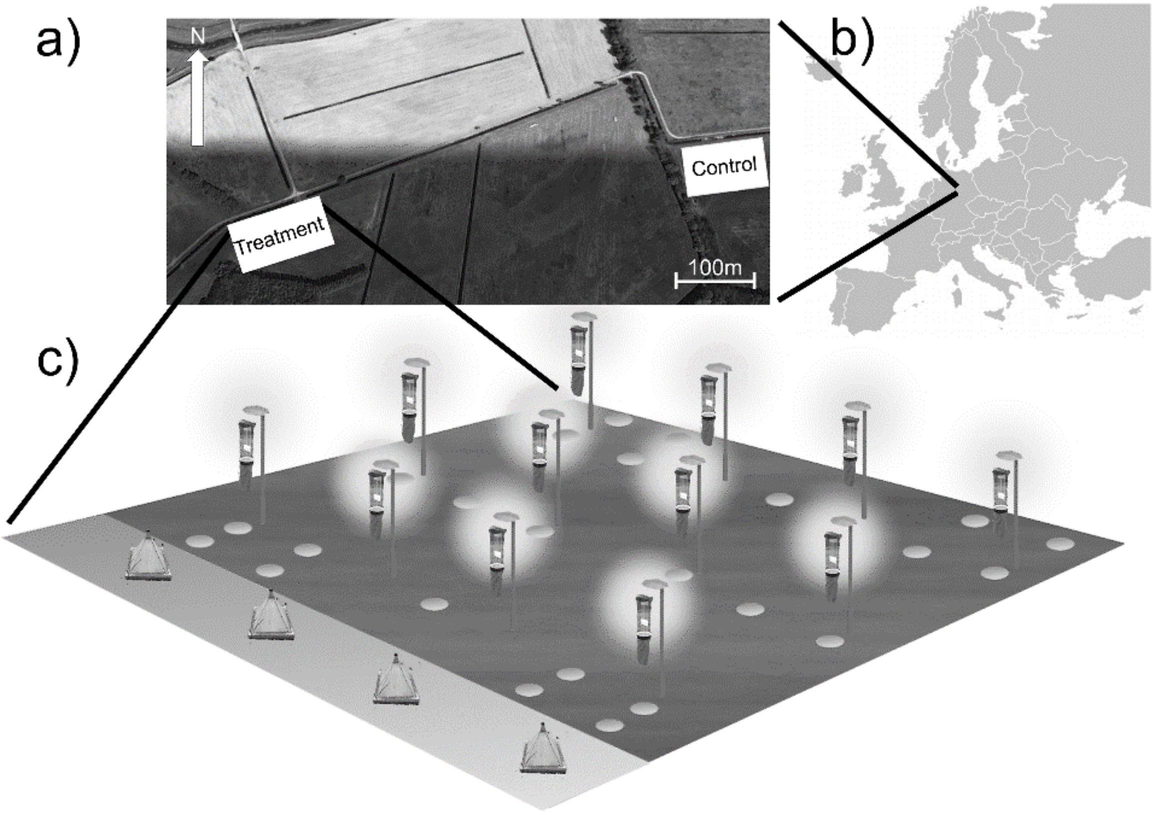


Figure 1. Study area in the Westhavelland region of Brandenburg, Germany depicting treatment and control sites (each 60 x 40 m) located along an agricultural drainage ditch (a, b). The lower schematic (c) depicts the treatment site with streetlamps and sampling traps (not to scale). Floating pyramidal emergence traps (*n* = 4) were placed adjacent to a lamp on the water surface of the drainage ditch. Air eclector traps (*n* = 12) were mounted below each lamp. Pitfall traps (*n* = 24) were placed on the ground in multiple locations (indicated by white circles). The control site had the identical experimental setup, the difference being that the street lights were not switched on.

3.3.2 Environmental conditions

Data collection started in June 2012. Weather stations at both sites continuously recorded air temperature, wind speed, humidity, and light intensity. Underwater probes continuously recorded water temperature, pH, oxygen, and chlorophyll-a in the drainage ditches. These data were used to ascertain continuous chemico-physical similarity between the two sites and obtain reference (baseline) values in the absence of illumination at the treatment site (Holzhauer et al. 2015).

3.3.3 Arthropod collection and identification

Insects were collected from both sites using identical procedures. Emerging aquatic insects were sampled using four floating pyramidal emergence traps (0.85 x 0.85m, 300-µm mesh) at each site. These were placed in the drainage ditch adjacent to the bank and one trap was placed directly in front of each streetlamp (Fig. 1c). Sampling was continuous for 128 - 192 hours but frequency varied according to emergence patterns. In 2012, sampling occurred weekly from May to August and monthly in September and October. In 2013, sampling occurred monthly from May to October except in July when sampling occurred weekly. Flying insects were collected using air eclector traps consisting of two perpendicular acrylic panels (each 204 mm × 500 mm × 3 mm) mounted above a collecting funnel and placed 0.5 m below each lamp. Ground-dwelling arthropods were collected using 48 pitfall traps, each consisting of a container (15-cm diameter) inserted in the ground with its rim at the soil surface. A transparent acrylic sheet was placed above each trap to prevent entering of precipitation and debris. Pitfall traps were positioned under and between streetlights at varying distances from the drainage ditch (Fig. 1c). Air eclector and pitfall trap sampling occurred bi-weekly from May to October in 2012 and in 2013. Sampling always occurred on rainless nights within one night of each half-moon phase (first and third quarter). In 2012, sampling was carried out at night and lasted from astronomical sunset to sunrise (8-14 hours depending on the season). In 2013, pitfall trap sampling was also conducted during the day (10 to 16 day-time hours) following the night sampling. All traps were fitted with collecting containers containing 70% ethanol for preservation (see Holzhauer et al. 2015 fur further details). Larval individuals were excluded from counts.

3.3.4 Experimental approach

The experiment was set up as a BACI design (Before-After, Control-Impact) (Stewart-Oaten et al. 1986), testing for differences in arthropod abundance and community composition between the dark control and the treatment, that was illuminated in a later phase only (Fig. 2). The period prior to ALAN addition, when both sites were dark, was May-July 2012. ALAN addition began in the treatment site in July 2012 and continued until the end of the study in October 2013. We made two statistical comparisons. The first compared the unlit and lit periods in 2012, i.e. May-July 2012 with August-October 2012 (Comparison I; see Fig. 2). The second comparison considered data from both years and compared the unlit period from May-July 2012 with the lit period from May-July 2013 to account for eventual changes in phenology affecting the first comparison (Comparison II; see Fig. 2). Statistical aspects of both comparisons are described more fully below. In addition to the BACI design, we also examined temporal patterns of insect abundance and ground-dwelling secondary consumer community composition from May until October 2013.

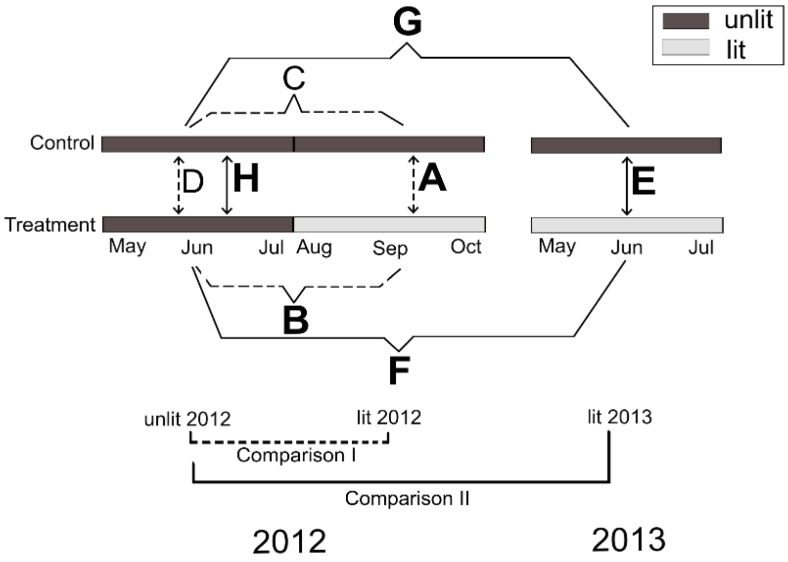


Figure 2. BACI (Before-After Control-Impact) design used for data analysis, indicating timing of illumination of the treatment site. Control and treatment sites prior to ALAN addition (unlit 2012) are compared during treatment illumination in 2012 (lit 2012) (Comparison I) and in 2013 (lit 2013) (Comparison II). Upper case letters indicate pairwise contrasts, used in the case of significance in the analysis of Comparison I (A-D) and Comparison II (E-H). Letters in bold indicate pairwise contrasts that were significant for at least one trap type in the analysis of the abundance (LME) or composition (perMANOVA).

3.3.5 Statistical analysis – environmental conditions

Treatment and control sites were compared using air temperature, humidity, water temperature, and dissolved oxygen. A one-way Generalized Least Squares (GLS) model with a fixed factor “site” (control, treatment) was performed for each of the BACI periods (see above) and for each month in 2013 using the lmne package (Pinheiro et al. 2015) for R (version 3.3.1) (R Core Team 2015). The analysis incorporated an autoregressive correlation structure of order 2 (corARMA = 2) to account for serial correlation of time series data. The data autocorrelation was tested for each variable using Durbin-Watson statistics in the car package (Fox et al. 2016) for R. The correlation structure suitability was tested using a likelihood-ratio test (see Holzhauer et al. 2015 for a similar approach).

3.3.6 Statistical analysis – arthropod abundance

For all analyses, arthropod abundance was standardised to the number of individuals caught per hour of trap operation (CPUE; catch per unit effort). For air eclector and pitfall traps, the two bi-weekly samplings in each month were pooled for analysis in order to reduce the number of zeros in the data matrix. For each trap type (emergence, air eclector, pitfall), differences in arthropod CPUE were examined using linear mixed effects (LME) models as implemented in the lme4 package (Bates et al. 2007) for R. For the BACI analysis, the fixed factors “site” (control, treatment) and “period” (unlit 2012 and lit 2012 in Comparison I; unit 2012 and lit 2013 in Comparison II, see Fig. 2) and their interaction, which tests the actual ALAN effect, were considered in each model. “traps” nested in “site” and “month” were considered as random factors to account for multiple observations and potential serial dependency. For the BACI comparisons of ground-dwelling secondary consumers, only nocturnal samples were analysed, as day-time data were lacking from 2012. High levels of precipitation and flooding caused malfunction of some pitfall traps in 2013 and we therefore only used data from positions where traps had remained functional at both sites. For each LME with a significant interaction, we performed contrast analysis as pairwise comparison using least-squares means (LSM) with the lsmeans package (Lenth 2016) for R. Comparisons for the BACI LME were performed between control and treatment site within the same period, and between two different periods within the same site (see Fig. 2).

For the analysis of temporal patterns in 2013, we used a two-way LME with the fixed factors “site” and “month” and their interaction, with “trap” nested in “site” as a random factor. For the analysis of the temporal patterns of ground-dwelling secondary consumers in 2013, where both diurnal and nocturnal samples were collected, we added “time of the day” (day, night) including all the possible fixed factor interaction. Subsequently, the temporal pattern LME (see above) was run separately for diurnal and nocturnal arthropods to examine both direct (night) and indirect (day) effects of ALAN. For each LME with a significant interaction, LSM pairwise contrast analysis comparison for the temporal pattern analysis were performed between sites for each month.

Each LME model with fixed factors (above) was compared with a reduced model (i.e. without the fixed factors) using a likelihood ratio test (Pinheiro and Bates 1995). The distribution of residuals was assessed using Wilk-Shapiro tests (Shapiro and Wilk 1965) and qq-plots (Wilk and Gnanadesikan 1968). To control for inflated false discovery rates, we used Benjamini-Hochberg corrected α-values for the pairwise contrast analyses (Waite and Campbell 2006).

3.3.7 Statistical analysis – community composition

Compositional differences among traps were computed as Bray-Curtis dissimilarities (Beals 1984). Prior to the analysis, we standardised the dataset on ground-dwelling secondary consumers using a chord transformation in order to increase the influence of rare species (Legendre and Gallagher 2001). Then a similar statistical approach to that used for the arthropod abundance (above) was also used for the multivariate analysis of composition. A two-way Permutational Multivariate Analysis of Variance (perMANOVA) was used to test for compositional dissimilarity among “sites” and “periods” including their interaction; this was done separately for the two BACI comparisons (Comparison I, Comparison II). Where a significant interaction effect was detected we performed one-way perMANOVA between each combination of sites and periods as a post-hoc test. To identify taxa driving differences in taxonomic composition between sites, we used similarity percentage (SIMPER) analysis (Clarke 1993) to produce a ranked list of taxa that cumulatively contributed more than 70% to site dissimilarity. Differences in taxonomic composition between sites were visualized using non-metric multidimensional scaling (nMDS).

To analyse temporal patterns of taxonomic composition we assessed compositional dissimilarity between diurnal and nocturnal assemblages and sites across months in 2013, the year for which both diurnal and nocturnal samplings were carried out. We first ran a randomized complete block (RCB) three-way perMANOVA (Wei et al. 2012) including interactions of the factors “site”, “month” and “time of the day” (day or night). Subsequently, to test for the temporal patterns in the impact of ALAN on community composition separately for diurnal and nocturnal arthropods, we performed an RCB two-way perMANOVA testing for variation in taxonomic composition among “sites” and “months” including interaction. In RCB perMANOVAs, “trap” was used as a blocking factor to account for individual trap variability and potential sample autocorrelation. When a significant “site” x “month” interaction was detected, we performed nMDS and one-way perMANOVAs (as post-hoc tests) for each month to plot and test compositional differences between sites. SIMPER analysis was conducted separately for diurnal and nocturnal arthropods in each month in which differences (after post-hoc perMANOVA) in taxonomic composition between sites were detected. All dissimilarity matrices were tested for homogeneity of multivariate dispersion (Anderson 2006). To control false discovery rate, we used the Benjamini-Hochberg procedure (Waite and Campbell 2006). We performed all compositional analyses using the vegan package (Oksanen et al. 2013) for R.

3.4 Results

3.4.1 Environmental conditions

Holzhauer et al. (2015) reported environmental conditions at the experimental sites prior to the start of ALAN treatment. When data from the following period including nocturnal illumination (i.e., July-October 2012 and May-October 2013) were added, air temperature and humidity did not differ between sites (Appendix S6, S7). During the experiment, mean daily water temperature was slightly higher in the control site than the treatment site. Water temperature was 1.8°C higher in the period prior to illumination (unlit 2012), 0.8°C higher during the period of illumination in 2012, and 1.3°C higher in 2013. In the temporal analysis in 2013, the difference between control and treatment site ranged from 0.4 - 2.6°C (Appendix S6, S7). Dissolved oxygen was 0.7 mg l^{-1} higher in the treatment site prior to illumination in 2012 and 1.32 mg l^{-1} higher after illumination in 2012, but was 1.5 mg l^{-1} lower than the control site in 2013 (Appendix S6, S7). In the temporal analysis in 2013, differences in dissolved oxygen ranged between 0.2 and 3.4 mg l^{-1} (Appendix S6, S7).

3.4.2 CPUE - Aquatic insect emergence

We collected 25 families of insects in emergence traps. Most individuals belonged to the Ephemeroptera (Baetidae, *Cloeon dipterum*), Trichoptera (5 families) or Diptera (17 families) (Taxon list in Appendix S8). The Model of BACI Comparison I (see Fig. 2) found no significant difference in emergence among periods and sites, i.e. before and after ALAN treatment in 2012 (Table 1, Fig. 3a). The model of BACI Comparison II (see Fig. 2), analysing data from the May-July periods of 2012 (unlit 2012) and 2013 (lit 2013), indicated significant differences in insect abundance (as CPUE) among sites and periods (Table 1, Fig. 3a). Significant pairwise contrasts showed that the lit treatment site had 2-fold higher insect CPUE than the control site in 2013 (Table 1, Fig. 3a, pairwise contrast E, see Fig. 2) and a 3-fold increased CPUE compared to prior to illumination in 2012 (Table 1, Fig. 3a; pairwise contrast F). There was no difference in CPUE between 2012 and 2013 at the control site (Fig. 3a), but CPUE was 0.6-fold lower at the treatment site than at the control site prior to illumination (Table 1, Fig. 3a; pairwise contrast H). The analysis of temporal patterns in 2013 indicated that the number of insects caught in the treatment site was 3 times higher in July when compared to the control site in the same month, but not different during other months (Table 2, Fig. 3b).

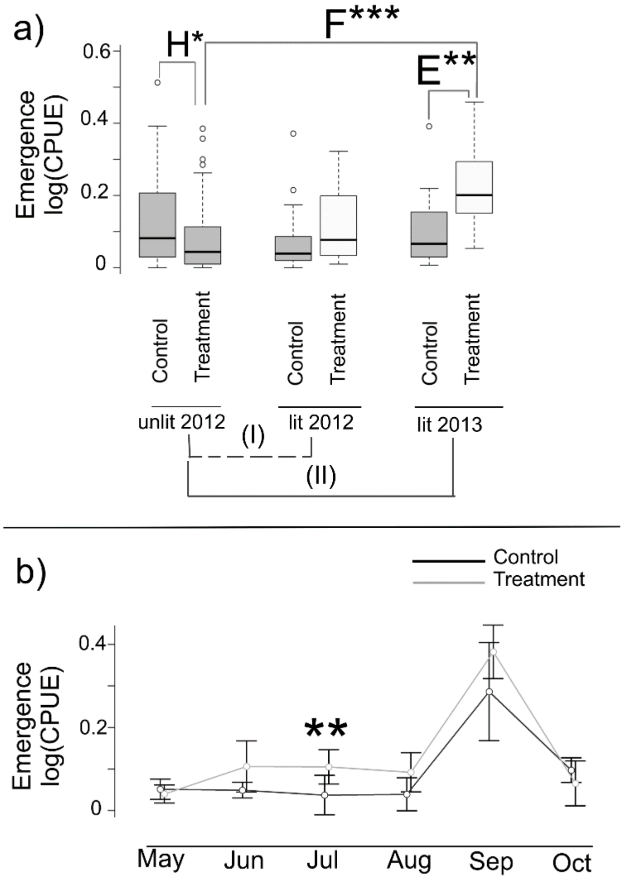


Figure 3. Abundance of emerging aquatic insects (CPUE) compared within and among years using a BACI design (a), and showing temporal patterns in 2013 (b). For the BACI analysis, control and treatment sites prior to ALAN addition (unlit 2012) were compared during experimental illumination in 2012 (lit 2012) (BACI comparison I) and in 2013 (lit 2013) (BACI comparison II). Significant pairwise contrasts are shown for comparison II (solid lines; E, F, H, see Fig. 2). Each box plot shows the median, lower, and upper quartiles; greatest and least values excluding outliers (whiskers); and outliers (circles). For the temporal analysis (b), abundance (CPUE) per month from May until October 2013 was compared between treatment and control traps. Asterisks indicate significant difference in the pairwise comparisons (*** = $p<0.001$; ** = $p<0.01$; * = $p<0.05$).

3.4.3 CPUE - Flying insects

We collected a total of 189 aquatic and terrestrial taxa in the air eclector traps. The majority of aquatic insects were Ephemeroptera and Diptera and the majority of terrestrial insects were Lepidoptera and Coleoptera (see Appendix S8 for a taxon list). For the BACI analyses, both linear models (Comparisons I, II) indicated significant differences among periods and sites for the abundance of flying aquatic insects (Table 1). Pairwise contrasts in 2012 (Comparison I) showed a clear increase of aquatic insect abundance at the lit treatment site: it was 9-fold higher than abundance at the control site, and 15-fold higher than abundance at the treatment site prior to illumination (Fig. 4a; Table 1; pairwise contrasts A, B in Fig. 2). In Comparison II abundance at the treatment site in 2013 was found to be 281-fold greater than at the control site in 2013 (pairwise contrast E) and 477-fold greater than abundance at the same site prior to illumination in 2012 (Fig. 4a; pairwise contrast F). There was no difference between control and treatment sites prior to illumination and no difference between 2012 and 2013 at the control site (Fig. 4a). Analysis of temporal patterns in 2013 indicated that insect abundance was always higher in the lit treatment site. This was most pronounced in July when CPUE was 460-fold higher than in the control site (Table 2, Fig. 4c). In other months, the difference ranged from 10- to 73-fold (Table 2, Fig. 4c).

Both models used in the BACI analysis (Comparison I, Comparison II) indicated significant variation among sites and periods for the abundance of flying terrestrial insects (Table 1). In 2012, after the start of ALAN treatment, the terrestrial insect abundance at the treatment site was 20-fold higher than at the control site and 33-fold higher than it had been at the treatment site prior to illumination (Fig. 4b; pairwise contrasts A, B in Fig. 2). There was no difference between sites prior to illumination or within the control site before and after illumination in 2012 (Fig. 4b). The treatment site in 2013 exhibited a 56-fold increase in abundance compared to the control site in 2013 and an 128-fold increased abundance compared to prior to illumination in 2012 (Fig. 4b; pairwise contrasts E, F). The analysis of temporal patterns of terrestrial insects in 2013 was also similar to that of the aquatic insects, with abundance always being 4- to 68-fold greater at the lit treatment site (Fig. 4d, Table 2).

The proportion of arthropods that were aquatic in origin did not differ among sites and periods in 2012 (Comparison I) (Appendix S9a), but differed among sites and periods in the comparison between years (Comparison II) (Table 1, Appendix S9a). In 2013 aquatic insects at the treatment site comprised 85% of the total catch, compared to 62% at the control site in the same year (i.e. ca. 1.3-fold greater) and 57% at the treatment site prior to illumination (i.e. ca. 1.5-fold greater) (Table 1; pairwise contrast E; F in Fig. 2; Appendix S9a). There was no difference in the proportion of aquatic insects between control and treatment sites in 2012 prior to illumination, nor between years at the control site (Appendix S9a). Analysis of temporal patterns indicated that the proportion of aquatic insects was consistently higher at the treatment site compared to the control: namely by factors 1.6, 1.6 and 4 in July, September and October, respectively (Table 2, Appendix S9b).

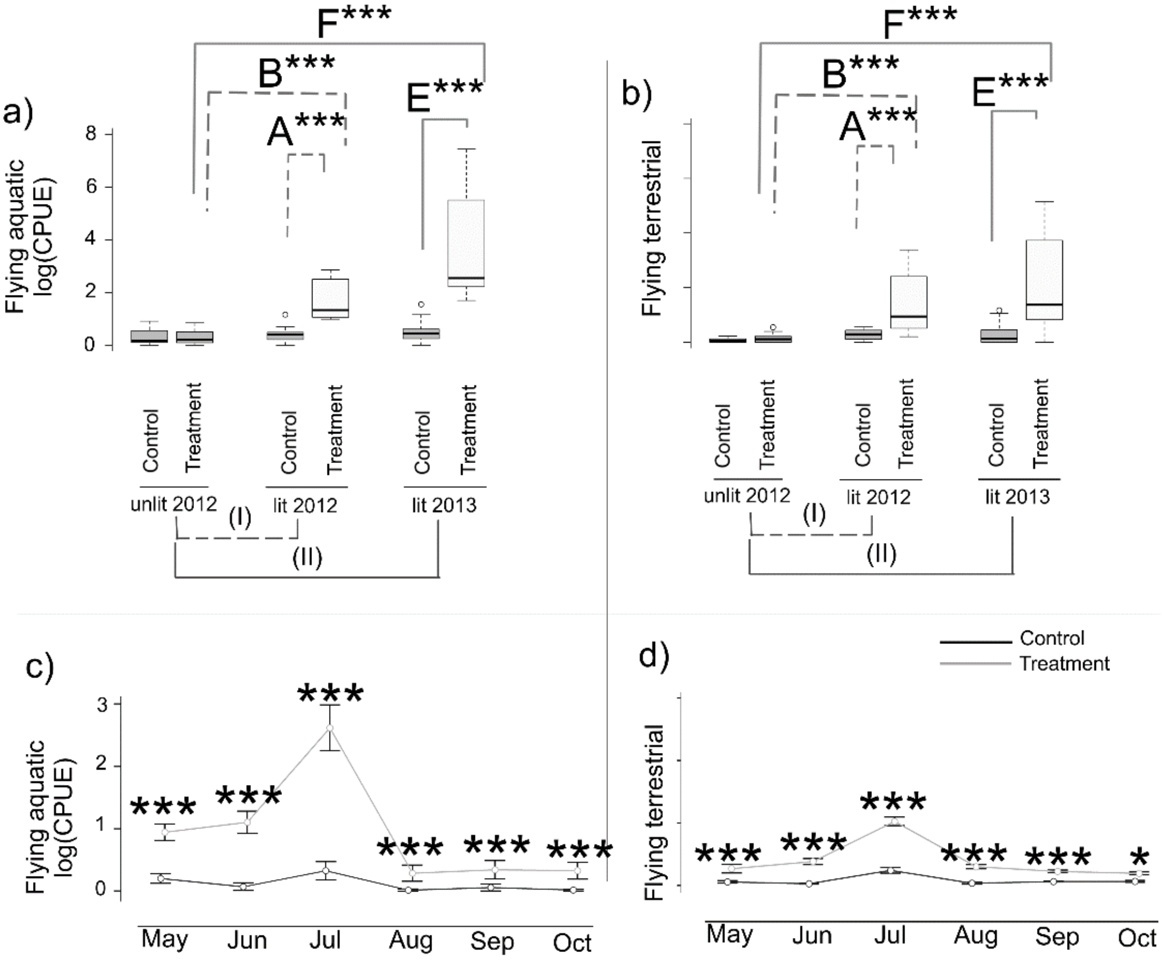


Figure 4. In the upper panels the number of individual/hour (CPUE) of flying aquatic (a) and terrestrial (b) insects caught in the air eclector traps are compared using a BACI design. Control and treatment sites prior to ALAN addition (unlit 2012) are compared during experimental illumination in 2012 (lit 2012) (Comparison I) and in 2013 (lit 2013) (Comparison II). Significant pairwise contrasts are shown for Comparison I (dashed lines; A, B; Fig. 2) and II (solid lines; E, F, Fig. 2). Each box plot shows the median, lower, and upper quartiles; greatest and least values excluding outliers (whiskers); and outliers (circles). The lower panels (c, d) depict temporal patterns of numbers of individuals/hour (CPUE) per month from May until October 2013 for the treatment and control site. Asterisks are used to indicate significant difference in the pairwise comparisons (*** = p<0.001; ** = p<0.01; * = p<0.05).

3.4.4 CPUE - Ground-dwelling arthropods

In total we collected 135 taxa of ground-dwelling arthropods in the pitfall traps. For primary consumers, there was no significant variation in CPUE among sites and periods in either BACI model (Comparisons I, II) (Table 1; Fig. 5a). In the analysis of temporal patterns in 2013 there was a difference in CPUE among sites and months (Table 2, Fig. 5c), with greater CPUE at the control site in August (7-fold) and September (15-fold) (Table 2, Fig. 5c). For secondary consumers, there was no difference in CPUE among sites and periods in the comparison between periods within 2012 (Comparison I) or between 2012 and 2013 (Comparison II) (Table1, Fig. 5b). Overall, more animals were collected in 2012 compared to 2013 (Comparison II) ($F_{1,96} = 250.93$, $p < 0.001$) (Fig. 5b). Analysis of temporal patterns in 2013 detected an interaction between site and time of the day ($F_{1, 216} = 5.53$, p = 0.02) in the abundance of secondary consumers. Subsequent analyses of the temporal patterns performed separately for time of the day (day, night) did not show a difference in the number of nocturnal arthropods between sites or months (Table 2, Fig. 5d), but there was a difference in abundances of the day-time samples (Table 2, Fig. 5e), with twice the number of secondary consumers at the treatment site compared to the control site during the day in July (Table 2, Fig. 5e).

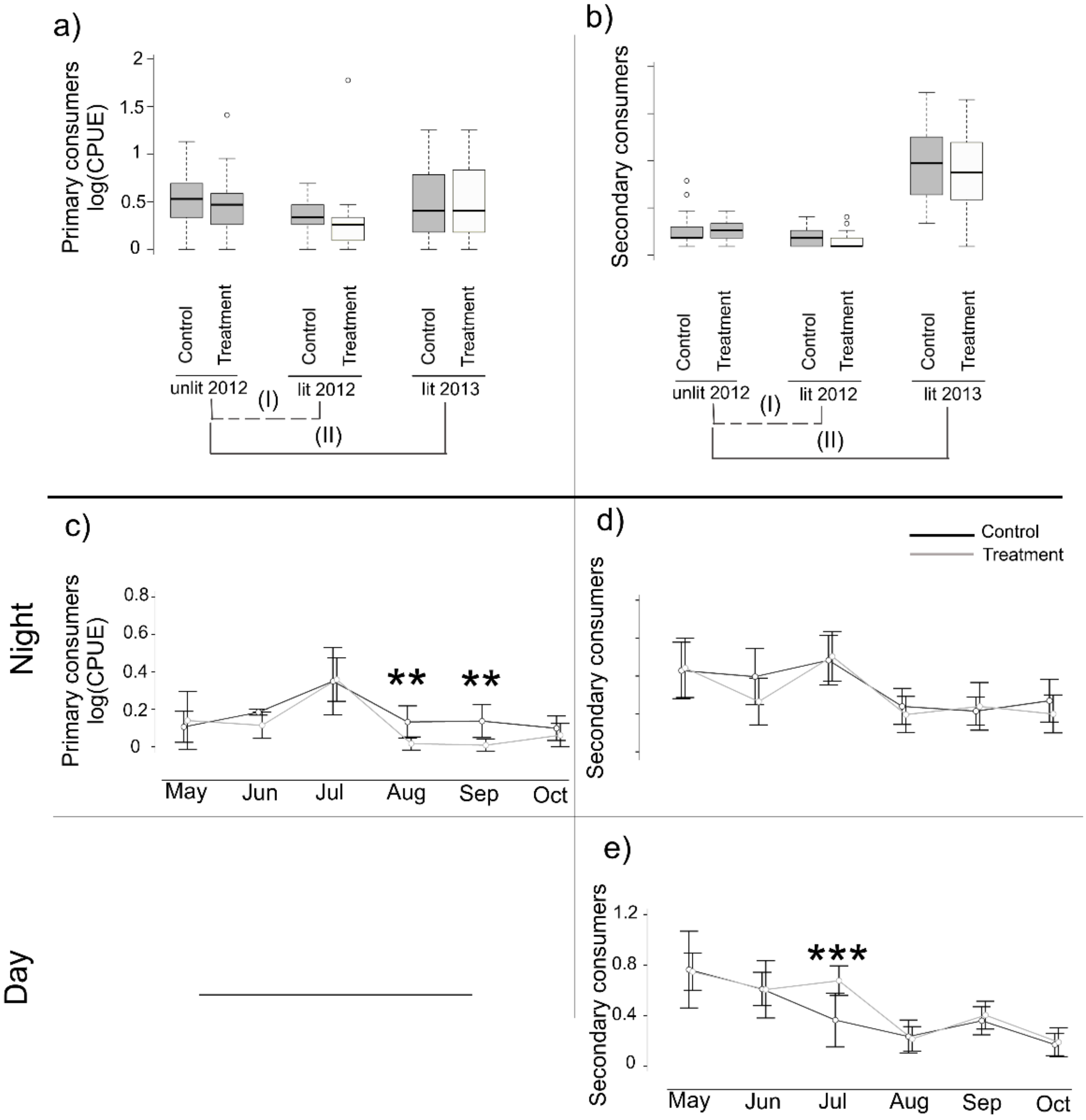


Figure 5. The abundance of individual (CPUE) of ground-dwelling primary (a) and secondary (b) consumers caught in pitfall traps were compared using a BACI design. Control and treatment sites prior to ALAN addition (unlit 2012) are compared during experimental illumination in 2012 (lit 2012) (Comparison I) and in 2013 (lit 2013) (Comparison II). Each box plot shows the median, lower, and upper quartiles; greatest and least values excluding outliers (whiskers); and outliers (circles). The lower panels (c, d, e) depicts temporal patterns of numbers of individuals/hour (CPUE) per month from May until October 2013 for the treatment and control site. For ground-dwelling secondary consumers, data are presented for nocturnal (d) and diurnal catches (e) separately. Asterisks are used to indicate significant difference in the pairwise comparisons (*** = p<0.001; ** = p<0.01; * = p<0.05).

Table 1. Arthropod abundance (CPUE) analysed using a BACI design. LME analysis was used to examine differences within and between sites prior to ALAN addition and during experimental illumination in 2012 (Comparison I; See also Fig. 2) and in 2013 (Comparison II). Analysis was conducted on emerging aquatic insects, flying (aquatic and terrestrial) insects, proportion of flying aquatic insects (on terrestrial) and ground-dwelling arthropods (primary, secondary consumers) for the three trap types. Results of the LME likelihood ratio test (χ²) and *F*-statistic for the site x period interaction are shown. Significant pairwise contrasts and *t*-statistic are shown for Comparison I (see Fig. 2; A = treatment vs control site in the "lit 2012" period; B = treatment site-"unlit 2012" period vs treatment site-"lit 2012" period) and for Comparison II (E = treatment site vs control site in "lit 2013" period; F = treatment site-"lit 2013" period vs treatment site-"unlit 2012" period; H = treatment vs control site in the "unlit 2012" period) (see Fig. 2). (*** = p<0.001; ** = p<0.01; * = p<0.05).

Trap type	Comparison	χ²	*F*-statistic (site x period)	Pairwise contrast	*t*-statistic
Emergence (emerging aquatic)	I	NS			
	II	23.19***	$F_{1,137}$=19.92***	E	t_{50}= 3.72***
				F	t_{136}= 4.65***
				H	t_{13}= -2.37*
Air eclector (flying aquatic)	I	86.73***	$F_{1,85}$=65.31***	A	t_{62}= -10.70***
				B	t_{73}= 6.02***
	II	154.68***	$F_{1,109}$=100.91***	E	t_{112}= -15.47***
				F	t_{112}= 13.01***
Air eclector (flying terrestrial)	I	88.08***	$F_{1,88}$=72.79***	A	t_{65}= -12.54***
				B	t_{97}= -2.99***
	II	84.02***	$F_{1,109}$=38.49***	E	t_{54}= -9.78***
				F	t_{96}= 8.23***
Air eclector (% flying aquatic)	I	NS			
	II	14.35**	$F_{1,112}$=8.96**	E	t_{55}= -3.12**
				F	t_{98}= 3.27**
Pitfall (primary consumers)	I	NS			
	II	NS			
Pitfall (secondary consumers)	I	NS			
	II	126.22***	NS		

Table 2. Analysis of the temporal patterns in the number of arthropods per hour (CPUE). Analysis was conducted on emerging aquatic insects, flying (aquatic and terrestrial) insects, proportion of aquatic flying insects (on terrestrial) and ground-dwelling arthropods (primary, secondary consumers) for the three trap types. LME was used to test differences among sites and months and their interaction. LME likelihood ratio test (χ^2) and *F*-statistic for site x period interaction are shown. LSM pairwise comparison and *t*-statistic are reported. Significance is reported as: *** = p<0.001; ** = p<0.01; * = p<0.05; NS = p>0.05. For ground-dwelling secondary consumer data are presented for diurnal and nocturnal catches (Time).

Trap type	Time	χ^2	*F*-statistic (site x month)	Pairwise (treatment vs control)	*t*-statistic
Emergence (emerging aquatic)	night	100.85***	$F_{5,60}$=2.49*	Jul	t_{40}=3.50**
Air eclector (flying aquatic)	night	439.99***	$F_{5,114}$=206.08***	May	t_{121}=11.66***
				Jun	t_{126}=15.60***
				Jul	t_{121}=36.07***
				Aug	t_{133}=3.8***
				Sep	t_{121}=4.49***
				Oct	t_{126}=4.69***
Air eclector (flying terrestrial)	night	375.54***	$F_{5,113}$=131.59***	May	t_{111}=4.60***
				Jun	t_{117}=7.67***
				Jul	t_{111}=27.6***
				Aug	t_{125}=5.24***
				Sep	t_{111}=3.46***
				Oct	t_{117}=2.50*
Air eclector (% flying aquatic)	night	97.08***	$F_{5,110}$=2.80*	Jul	t_{139}=3.34***
				Sep	t_{139}=2.51*
				Oct	t_{141}=5.07***
Pitfall (primary consumers)	night	99.35***	$F_{5,116}$=2.61*	Aug	t_{129}=2.69**
				Sep	t_{129}=3.07**
Pitfall (secondary consumers)	night	82.50***	NS		
	day	151.08***	$F_{5,99}$=5.04***	Jul	t_{121}=4.5***

3.4.5 Community composition of ground-dwelling secondary consumers

There was no significant difference in the composition of ground-dwelling secondary consumers between sites or periods in 2012 (Comparison I) (Fig. 6a); however, there was a difference when sites and periods were compared between 2012 and 2013 (Comparison II) ($F_{1,96}$ = 2.36, p = 0.018) (Fig. 6b). Community composition differed between treatment and control sites in 2013 ($F_{1,48}$ = 6.69, p < 0.001) (Fig. 6b, pairwise contrast E in Fig. 2) and between the treatment site in 2013 and prior to illumination ($F_{1,58}$ = 12.54, p < 0.001) (Fig. 6b, pairwise contrast F). Composition also differed between 2012 and 2013 ($F_{1,43}$ = 8.71, p < 0.001) at the control site (Fig. 6b, pairwise contrast G). No difference in composition was observed between the control and treatment sites prior to illumination (Fig. 6a).

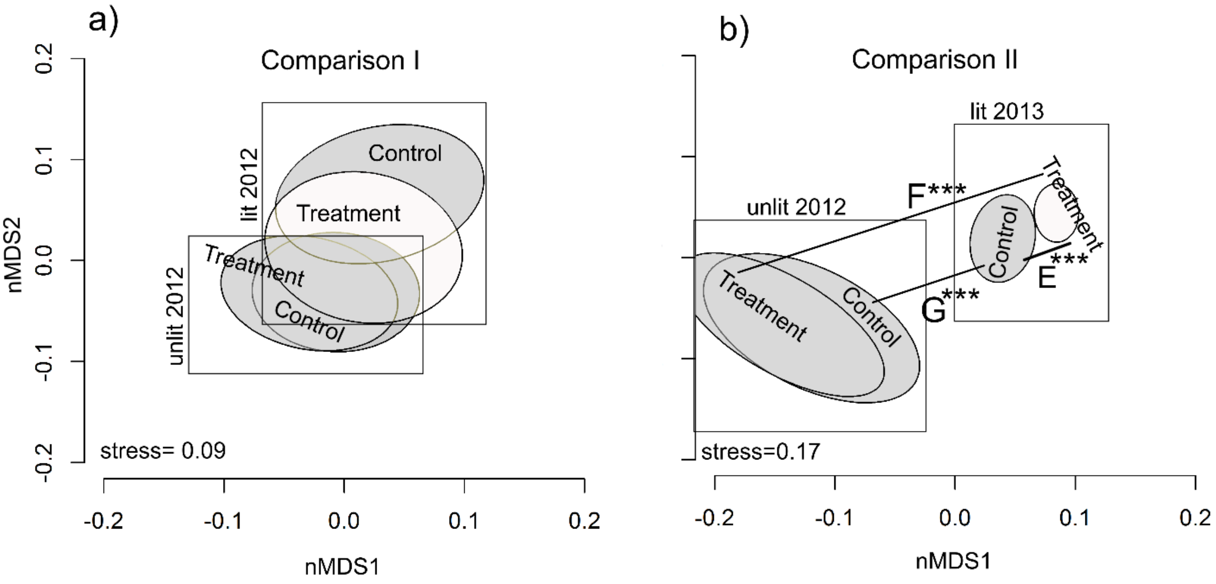


Figure 6. BACI analysis of the composition of the ground-dwelling secondary consumer community illustrated with non-metric multidimensional scaling (nMDS). Community composition of treatment and control traps is plotted separately for BACI Comparison I (a) and II (b). Ellipses represent 95% confidence areas before and after artificial illumination started. Significant pairwise contrasts are shown for Comparison II (solid lines; E, F, G, see Fig. 2) (perMANOVA: *** = p<0.001).

A similarity percentage analysis (SIMPER) was used to identify taxa that were contributing to the compositional dissimilarity during the night. In 2013, Opilionidae (harvestmen), Linyphiidae (sheet spiders), *Pachygnatha clercki* (a long-jawed spider), and *Trochosa ruricola* (a wolf spider) all were more abundant in the treatment site (Table. 3). Conversely, *Pirata piraticus* (a wolf spider) Staphylinidae (rove beetles), and *Pterostichus nigrita* and *Agonum duftschmidi* (both ground beetles) were more abundant at the control site (see Table 3). Taxa that differed between treatment and control in 2013, also differed between post and pre-illumination periods at the treatment site (see Table 3). A difference in composition was also found at the control site between 2012 and 2013 (see Table 3).

In the analysis of temporal patterns, community composition of secondary consumers differed among sites, months, and times of the day (three-way interaction: $F_{1,\ 229} = 2.52$, $p = 0.009$). Differences in composition were found among sites and months for the arthropods collected during the night ($F_{5,104} = 1.64$, $p = 0.01$) and during the day ($F_{5,110} = 1.89$, $p < 0.001$). One-way perMANOVAs for each month uncovered differences between the control and treatment site for nocturnal communities in May ($F_{1,\ 21} = 3.59$, $p = 0.002$) and July ($F_{1,\ 17} = 7.10$, $p < 0.001$) (Fig. 7a). Similarly, differences in composition between sites were found for diurnal communities in May ($F_{1,\ 20} = 4.11$, $p = 0.002$), July ($F_{1,\ 17} = 4.82$, $p < 0.001$) and September ($F_{1,\ 22} = 3.32$, $p < 0.001$) (Fig. 7b). Opiliones were more abundant in traps at the treatment site compared to the control site during the nights in May and July (Table 4). Linyphiidae, *Arctosa leopardus, Alopecosa sp. and Throcosa sp.* were more abundant in traps at the treatment site during the days as well as the nights in May and June. For *Pachygnatha clercki*, this was also the case in September (Table 4). The two beetles *Agonum sp.* and *Pterostichus nigrita,* the wolf spider *Pirata piraticus* and the rove beetles *Staphylinidae* were more abundant at the control site than at the treatment site throughout the year during day and night (Table 4).

The wolf spiders *Pardosa paludicola, P. monticola, P. amentata, P. pullata*, and the carrion beetle *Silpha obscura* all contributed to the compositional dissimilarity between the treatment and control sites during the day. *Pardosa* spp. were more abundant in the treatment site in May and June with the exception of *Pardosa prativaga*, that was more abundant at the control site in May and June and at the treatment site in July and September. *Silpha obscura* was more abundant in September and October. In contrast, the ground beetle *Carabus granulatus* was more abundant at the control site in June and July (see Table 4).

Table 3. Mean abundance of the ground-dwelling secondary consumer taxa that contributed up to 70% of dissimilarity (similarity percentages analysis, SIMPER) in composition among sites and periods. The analysis was conducted on the BACI pairwise contrasts for Comparison II ("unlit 2012" period – "lit 2013" period) in which community composition was different (after perMANOVA). Taxa are listed according to the contribution percentage to the average site dissimilarity.

Pairwise contrasts (site x period)	Taxa	Contribution (%)	mean CPUE 1	mean CPUE 2
			Treatment-lit 2013	Control-lit 2013
Treatment-lit 2013/ Control-lit 2013	Opiliones	0.12	0.30	0.07
	Linyphiidae	0.11	0.28	0.09
	Pirata piraticus	0.09	0.01	0.20
	Pachygnatha clercki	0.09	0.23	0.10
	Staphylinidae	0.06	0.01	0.13
	Pterostichus nigrita	0.05	0.00	0.11
	Agonum duftschmidi	0.04	0.00	0.09
	Trochosa ruricola	0.03	0.06	0.04
			Treatment-lit 2013	Treatment-unlit 2012
Treatment-lit 2013/ Treatment-unlit 2012	Linyphiidae	0.20	0.28	0.00
	Opiliones	0.20	0.30	0.00
	Pachygnatha clercki	0.15	0.23	0.02
	Trochosa ruricola	0.04	0.06	0.00
	Arctosa leopardus	0.04	0.05	0.00
	Trochosa terricola	0.03	0.03	0.00
			Control-unlit 2012	Control-lit 2013
Control-unlit 2012/ Control-lit 2013	*Pirata piraticus*	0.15	0.02	0.20
	Staphylinidae	0.10	0.03	0.13
	Pterostichus nigrita	0.08	0.00	0.11
	Pachygnatha clercki	0.07	0.02	0.10
	Agonum duftschmidi	0.07	0.00	0.09
	Linyphiidae	0.06	0.00	0.09
	Opiliones	0.05	0.02	0.07

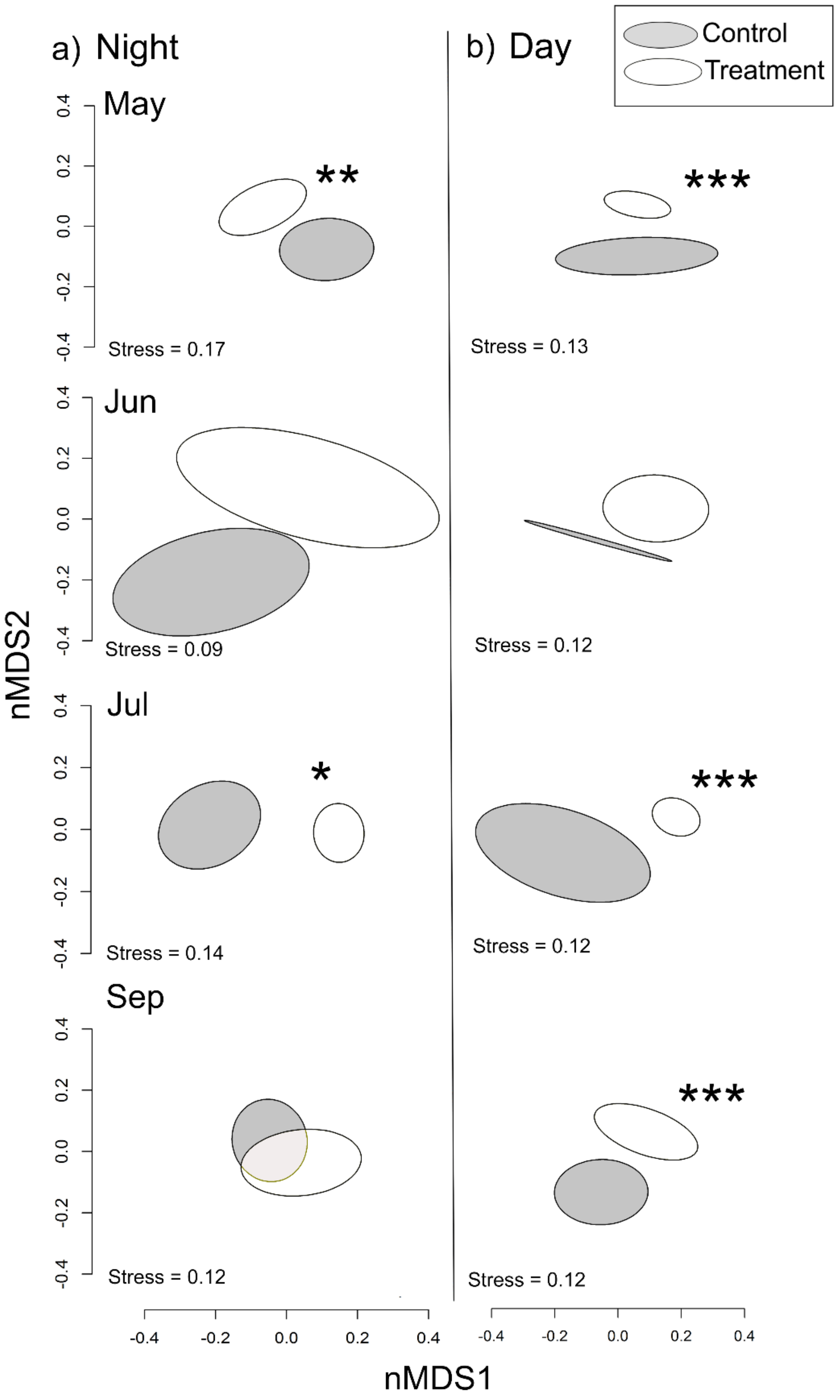


Figure 7. Temporal analysis of the community composition of ground-dwelling secondary consumers using non-metric multidimensional scaling (nMDS). Only months in 2013 with a significant difference between the treatment (lit) and the control traps in nocturnal (a) and/or diurnal (b) communities are shown (perMANOVA: *** = p<0.001; ** = p<0.01; * = p<0.05). Ellipses represent 95% confidence intervals for the treatment and control site.

Table 4. Taxa that contributed to the dissimilarity in community composition (SIMPER analysis) for diurnal and nocturnal ground-dwelling secondary consumers in the analysis of the temporal patterns performed in 2013. Only months in which communities were significantly different between the control and treatment site are shown (perMANOVA $p<0.05$). Taxa are listed according to light preference (from positive to negative). Taxa more abundant in the treatment site are shown in normal font and in the control site in bold font. Values indicate % contribution to the average site dissimilarity, with the ratio of change in abundance (higher) given in parentheses, i.e. *Pardosa paludicola* abundance was 2-fold higher in the treatment traps compared to the control traps during the day.

	May		June	July		September
Taxa	Night	Day	Day	Night	Day	Day
Pardosa paludicola		3.0 (2)	6.5 (6)		3.2 (11)	
Pardosa monticola		3.3 (19)	2.5 (9)			
Pardosa amentata		2.8 (1)				
Pardosa pullata			2.2 (1)			
Arctosa leopardus	5.4 (13)	2.1 (1)	2.0 (8)			
Alopecosa sp.		3.6 (11)				
Trochosa sp.	6.8 (2)					
Opiliones	6.5 (2)			14.4 (8)		
Linyphiidae	7.4 (3)	4.8 (3)		13.3 (3)	10.5 (3)	7.1 (5)
Pachygnatha clercki	9.3 (2)			8.9 (4)	10.3 (31)	5.2 (1)
Silpha obscura					5.2 (15)	7.2 (4)
Poecillus versicolor		2.6 (2)	**6.9 (2)**		3.2 (14)	
Staphylinidae	**4.1 (7)**			**6.1 (9)**		**5.6 (5)**
Pardosa prativaga		**21.6 (3)**	**11.8 (1)**		5.6 (1)	9.9 (2)
Pterostichus nigrita	**6.2 (12)**					**4.2 (7)**
Carabus granulatus			**2.6 (4)**		**3.5 (1)**	
Pirata piraticus	**12.6 (29)**			**7.4 (20)**		**6.6 (4)**
Agonum duftschmidi		**2.3 (3)**	**2.6 (3)**	**9.7 (14)**	**6.7 (7)**	**4.3 (8)**

3.5 Discussion

We experimentally introduced ALAN to a riparian ecosystem and assessed changes to aquatic-terrestrial subsidy dynamics and to the receiving consumer community. Artificial illumination at night increased the flux of aquatic insects into the riparian area through an increase in emergence directly under lamps as well as increased attraction of aquatic insects from nearby areas to light. The riparian community of ground-dwelling predators and scavengers was altered in the lit area and the large increase in input of freshwater-derived prey was likely the primary driver of this change (Fig. 8).

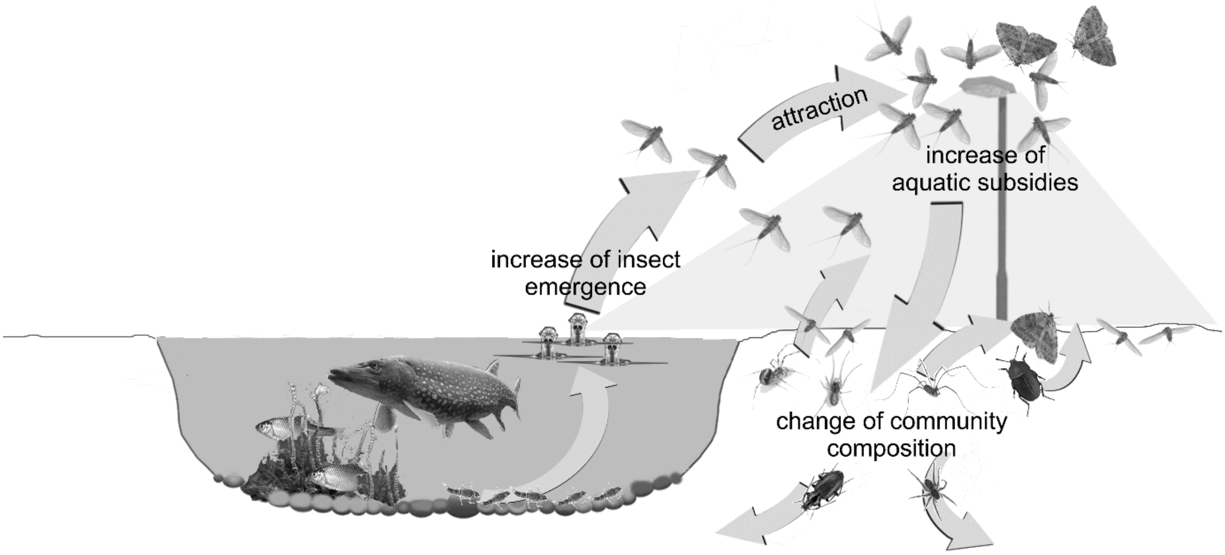


Figure 8. Conceptual figure depicting how artificial light at night (ALAN) increases the flux of aquatic insects into the riparian area through an increase in emergence under lamps and increased attraction of aquatic insects to light. The community of riparian ground-dwelling predators and scavengers is altered in the lit area and some night-active riparian spiders extended their activity into the day. Both likely were the result of the large increase in input of freshwater-derived prey.

Aquatic insect emergence increased in the artificially lit waterbody in 2013 compared to 2012, but there was no such change in the control site. The change was such that in the second year of illumination (2013), twice as many insects emerged from the lit site than the control site. Photoperiod and water temperature are important cues for aquatic insect emergence (Ward and Stanford 1982). Increased water temperature typically results in faster growth rates and early adult emergence (Ward and Stanford 1982, Harper and Peckarsky 2006). Theoretically, this could be also the case in our experiment because the mean daily water temperature was slightly higher (1.7 °C) in the control site compared to the ALAN-treated site. However, when applying the rectangle method of Lee and Watanabe (1999) this results in an increase of only 7 degree days, and there was no shift in the timing of emergence which peaked in September in both sites. Consequently, the minimal difference is unlikely to explain such a change in abundance.

Oxygen concentrations can also influence insect emergence. Connolly et al. (2004) found that mayfly emergence was reduced by 60% in hypoxic (25-35% saturation) compared to normoxic (95 – 100% saturation) water. This oxygen-sensitive taxon was the most abundant taxon in our emergence traps. It was thus surprising that we found greater emergence in the lit section of the ditch, despite the local hypoxic conditions, particularly in July (see Appendix S6, S7). Food availability (e.g. periphyton for primary consumers) is another factor affecting insect emergence. Péry et al. (2002) observed that food limitation (0.10 – 0.15 mg/larva/day) reduced *Chironomus riparius* emergence by 15% compared to individuals fed ad libitum. However, a study conducted in 2014 showed that periphyton biomass is the same in lit and control sites (unpublished data, M. Grubisic).

Another possibility is that ALAN increased aquatic insect emergence indirectly by reducing fish predation. Increased illumination is generally associated with increased predation risk (Cerri and Fraser 1983). In illuminated conditions, diurnal piscivorous fish can extend their hunting activity at night, increasing predation pressure on smaller fish (Becker et al. 2013). Invertivorous fish, including *Gasterosteus aculeatus* (three-spined stickleback), *Rhodeus amarus* (European bitterling) and young *Perca fluviatilis* (European perch), as well as piscivorous visual predators such as *Esox lucius* (Northern pike) and adults of *P. fluviatilis*, were abundant in the ditches of the study area (A. Manfrin, pers. obs.). The invertivores have to balance predator-avoidance and feeding efficiency (Fraser and Metcalfe 1997, Nightingale et al. 2006) and this may have reduced predation on aquatic invertebrate communities, leading to increased abundance and emergence (Fig. 8). Lee at al. (2013) attributed reduced emergence in *Cloeon dipterum* (the most abundant species in our emergence traps) to increased fish predation in a wetland. At the same time, ALAN might also have aggregated the immature insects (still in the water) near the lights. Because the emergence traps were located directly under lights, this may have resulted in higher local densities and therefore more individuals in the emergence traps. We did not measure benthic densities of immature aquatic stages in the two ditches. It is, therefore, not possible to identify a clear mechanistic explanation for the higher aquatic insect emergence in the artificially lit waterbody. Nonetheless, the effect was present within one year after the start of the exposure to light suggesting increased survival and higher population densities.

The addition of illumination attracted a large number of aquatic and terrestrial flying insects to the air eclector traps (Fig. 8). Light sources function as an ecological trap (van Langevelde et al. 2011, Degen et al. 2016) for many insects that are attracted to them. This occurs especially during swarming events in which very large numbers of individuals can be attracted to artificial light sources (Horváth et al. 2009). If not killed immediately, insects are often unable to disperse and migrate elsewhere (Perkin et al. 2011, Degen et al. 2016). The majority of insects collected in the lit traps were of aquatic origin, suggesting that aquatic insects might be more attracted and thus vulnerable to ALAN than terrestrial insects (see also Perkin et al. 2014a). Several studies have shown how artificial illumination disrupts dispersal patterns in arthropods, confounding natural sources of orientation (e.g. moonlight) and attracting phototactic insects (Horváth et al. 2011, Meyer and Sullivan 2013). In particular, aquatic insects perceive polarised light on the water as an important indicator of suitable egg-laying habitat and an important orientation cue (Horváth et al. 2009, Perkin et al. 2014a), further indicating the risk that ALAN pose for this group.

We found no evidence for a consistent effect of ALAN on ground-dwelling primary consumers. The only observed difference was a higher CPUE in the control site in August and September 2013. The general pattern of a peak in abundance in July, followed by a decrease, was observed in both lit and control traps. Secondary consumer abundance was similar in the dark and lit sites. We expected ground-dwelling predators and scavengers to be attracted by the large number of flying insects at lit traps. This might constitute “easy” prey, present as exhausted or dead individuals on the ground (Eisenbeis et al. 2006, Perkin et al. 2011). The only exception was observed in July 2013 when ground-dwelling secondary consumers were more abundant in the treatment than in the control site in the diurnal catch. This may have been related to the large number of flying insects at the treatment site the night before.

In contrast to abundance patterns, community composition of secondary consumers differed between lit and control sites at night. We found specific predators and scavengers to be more abundant in lit sites (s.a. Davies et al. 2012). We observed an increased abundance of Tetragnathidae spiders and Opiliones (harvestmen) at night under ALAN. Interestingly, Meyer and Sullivan (2013) reported reduced abundance for Tetragnathidae due to ALAN. This difference is probably explained by the fact that Tetragnathidae in our study were dominated by *Pachygnatha clercki*. The ecology of this species is not typical for Tetragnathidae. Most species of this family are sit-and-wait predators that build webs, but adults of *P. clercki* are night-active visual hunters and do not use webs. *P. clercki* also has a tapetum in the secondary eyes that increases visual efficiency at low light levels (Land 1985). The increase in abundance of a species showing these traits in a lit site at night suggests that it is able to make efficient use of the light levels provided by ALAN. An exception was the wolf spider *Pirata piraticus*, which responded negatively to ALAN. This species hunts on banks or directly on the water surface. Under artificial light it may therefore be easily preyed upon by visual predators (e.g. birds, bats, toads). In contrast to our observations of spiders, most ground beetles (e.g. *Agonum duftschmidi, Pterostichus nigrita, Carabus granulatus*) had reduced abundance in the lit site. These taxa may have been directly repelled by light, or may have suffered from increased predation from, or competition with, the abundant ground spiders (Punzo et al. 2006).

Our results also provide evidence for an effect of artificial illumination that persists during the day and affects diurnal communities (s.a. Davies et al. 2012). The response of the diurnal community likely reflects an increased availability of prey in artificially illuminated areas. The night-active spiders Linyphiidae*, P. clercki, Trochosa* sp., *A. leopardus,* and *Alopecosa* sp. (the latter three are wolf spiders) and the night-active scavenger carrion beetle, *Silpha obscura*, all extended their activity into the day in the treatment site. We conclude that these predators may have benefited from the presence of exhausted or dead insects that were attracted to the lights the night before. These observations indicate that the effect of artificial illumination at night can persist into the following day and affect local diurnal communities.

The presence of artificial light adjacent to water bodies may affect the distance that organisms move away from the water and therefore the spatial scale of the aquatic-terrestrial subsidy signature of a river landscape (Perkin et al. 2011, Gurnell et al. 2016). Our traps were located 3 m from the water and collected 85-fold more aquatic insects compared to unlit controls. A recent review found the density of aquatic insects is reduced by 50% within the first 1.5 m from the water's edge (Muehlbauer et al., 2014).

Given the global abundance of street lights along streams and rivers and along the shores of lakes, reservoirs, and wetlands, ALAN potentially can change cross-ecosystem fluxes at regional and global scales. Due to the important role of aquatic subsidies to consumers in recipient ecosystems, the impact of artificial illumination has to be considered as a relevant stressor, in urban and landscape planning. It is highly important to include mitigation measures into new lighting concepts to address potential ecological impacts on cross-ecosystem fluxes. This requires substantive multidisciplinary efforts by landscape and urban planners, lighting engineers, and terrestrial and aquatic ecologists to advance scientific understanding and to use these advances to improve restoration and management of aquatic-terrestrial habitats. We suggest the installation of artificial lights directly adjacent to stream riverbanks should be carefully designed, for example, by establishing adequate spatial and temporal riparian buffers in which natural dynamics of movement and dispersal of both aquatic and terrestrial organisms are protected. ALAN needs to be only directed to where is needed, in the lowest intensity required for its use and only when necessary (s.a. Schroer and Hölker 2016).

Our study design allowed us to assess ALAN in the field at the aquatic-terrestrial ecotone. The experimental erection of street lights in a previously ALAN-naïve area allowed us (1) to disentangle the effects of ALAN from other aspects of urbanization such as pollution, noise, and habitat alteration that confounds most studies; and (2) to minimize the effects of potential long-term adaptations that may have already occurred in areas that have been lit for many generations. Several findings are in agreement with previous and ongoing work in other ecosystems and other experiments, strongly suggesting that there are consistent patterns of response in freshwater and terrestrial insect communities. Our observations have raised new research questions in the field that can now be studied mechanistically, at smaller scales. For example, we used high-pressure sodium lamps which are considered to be relatively 'insect friendly' (Eisenbeis et al. 2006). The current global shift to the use of LED lamps, with peaks in spectral white-blue, may have even greater effects on nocturnal invertebrates given their sensitivity to short wavelength light (Langevelde 2011, van Grunsven et al 2014, Pawson and Bader 2014), which would have major implications for conservation biology, as well as for lighting policy and landscape planning.

3.6 Acknowledgements

We thank Stefan Heller, Maja Grubisic, Stephanie Holzhauer, Sibylle Schroer, Martin Oehlert, Christian Schomaker, Liliana Lehmann and Babette Pohlmann for help during the experiments. We thank Francesca Pilotto, Kirsten Pohlmann, Kate Laskowski, Ulrike Scharfenberger and Thomas Mehner for advice on statistical analysis. This work was 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 (FKZ 3514821700). S. Larsen was supported by an individual fellowship from the German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany.

3.7 References

Anderson, M. J. 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics **62**: 245-253.

Bartels, P., J. Cucherousset, K. Steger, P. Eklöv, L. J. Tranvik, and H. Hillebrand. 2012. Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer resource dynamics. Ecology **93**: 1173-1182.

Bates, D., D. Sarkar, M. D. Bates, and L. Matrix. 2007. The lme4 package. R package version 2: 1-74.

Beals, E. W. 1984. Bray-Curtis ordination: an effective strategy for analysis of multivariate ecological data. Advances in Ecological Research **14**: 1-55.

Becker, A., A. K. Whitfield, P. D. Cowley, J. Järnegren, and T. F. Næsje. 2013. Potential effects of artificial light associated with anthropogenic infrastructure on the abundance and foraging behaviour of estuary-associated fishes. Journal of Applied Ecology **50**: 43-50.

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

Cerri, R. D., and D. F. Fraser. 1983. Predation and risk in foraging minnows: balancing conflicting demands. American Naturalist **121**: 552-561.

Clarke K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology **18**: 117-143.

Connolly, N., M. Crossland, and R. Pearson. 2004. Effect of low dissolved oxygen on survival, emergence, and drift of tropical stream macroinvertebrates. Journal of the North American Benthological Society **23**: 251-270.

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

Degen, T., O. Mitesser, E. K. Perkin, N. S. Weiß, M. Oehlert, E. Mattig, and F. Hölker. 2016. Street lighting: sex-independent impacts on moth movement. Journal of Animal Ecology **85**: 1352-1360.

Eisenbeis, G., C. Rich, and T. Longcore. 2006. Artificial night lighting and insects: attraction of insects to streetlamps in a rural setting in Germany. Pages 191-198 *in* Rich G. C. and Longcore T. editors. Ecological consequences of artificial night lighting. Island Press, Washington DC, USA.

Fox, J., S. Weisberg, D. Adler, D. Bates, G. Baud-Bovy, S. Ellison, D. Firth, M. Friendly, G. Gorjanc, and S. Graves. 2016. Package ‘car’. See http://cran-r.project.org/web/packages/car/car.pdf.

Fraser, N., and N. Metcalfe. 1997. The costs of becoming nocturnal: feeding efficiency in relation to light intensity in juvenile Atlantic salmon. Functional Ecology **11**: 385-391.

Gaston, K. J., M. E. Visser, and F. Hölker. 2015. The biological impacts of artificial light at night: the research challenge. Philosophical Transactions of the Royal Society of London B **370**, 20140133.

Gurnell, A. M., W. Bertoldi, K. Tockner, G. Wharton, and G. Zolezzi. 2016. How large is a river? Conceptualizing river landscape signatures and envelopes in four dimensions. Wiley Interdisciplinary Reviews: Water **3**: 313-325.

Harper, M. P., and B. L. Peckarsky. 2006. Emergence cues of a mayfly in a high-altitude stream ecosystem: potential response to climate change. Ecological Applications **16**: 612-621.

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

Hölker, F., C. Wurzbacher, C. Weißenborn, M. T. Monaghan, S. I. Holzhauer, and K. Premke. 2015. Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. Philosophical Transactions of the Royal Society of London B **370**, 20140130.

Holzhauer, S. I., S. Franke, C. Kyba, A. Manfrin, R. Klenke, C. C. Voigt, D. Lewanzik, M. Oehlert, M. T. Monaghan, S. Schneider, S. Heller, H. Kuechly, A. Brüning, A.C. Honnen, and F. Hölker. 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**: 1-22.

Horváth, G., G. Kriska, P. Malik, and B. Robertson. 2009. Polarized light pollution: a new kind of ecological photopollution. Frontiers in Ecology and the Environment **7**: 317-325.

Horváth, G., A. Móra, B. Bernáth, and G. Kriska. 2011. Polarotaxis in non-biting midges: female chironomids are attracted to horizontally polarized light. Physiology and Behavior **104**: 1010-1015.

International Dark Sky Association (IDA). International Dark Sky Reserves: Westhavelland (Germany). Available online: http://darksky.org/ idsp /reserves/ westhavelland/ (accessed on 13 November 2015).

Kurvers, R. H., and F. Hölker. 2015. Bright nights and social interactions: a neglected issue. Behavioral Ecology **26**: 334-339.

Kummu, M., H. De 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**: 1-13.

Land, M. F. 1985. The morphology and optics of spider eyes. Pages 53-78 *in* Land M. F. editors. Neurobiology of arachnids. Springer Berlin, Germany.

Lee, C.Y., D. G. Kim, M. J. Baek, L. J. Choe, Y. J. Bae. 2013. Life history and emergence pattern of *Cloeon dipterum* (Ephemeroptera: Baetidae) in Korea. Environmental Entomology **42**: 1149-1156.

Lee, S., and N. Watanabe. 1999. Comparisons of temperature-related life histories in two ephemerid mayflies (*Ephemera separigata* and *E. strigata*: Ephemeridae, Ephemeroptera, Insecta) from a mountain stream in Korea. Korean Journal of Limnology **32**: 253-260.

Legendre, P., and E. D. Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia **129**: 271-280.

Lenth, R. V. 2016. Least-squares means: the R Package lsmeans. Journal of Statistical Software **69**: 1-33.

Longcore, T., and C. Rich. 2004. Ecological light pollution. Frontiers in Ecology and the Environment **2**: 191-198.

Marczak, L. B., and J. S. Richardson. 2007. Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. Journal of Animal Ecology **76**: 687-694.

Mehner, T., J. Ihlau, H. Dörner, and F. Hölker. 2005. Can feeding of fish on terrestrial insects subsidize the nutrient pool of lakes? Limnology and Oceanography **50**: 2022-2031.

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

Moore, M. V., S. M. Pierce, H. M. Walsh, S. K. Kvalvik, and J. D. Lim. 2001. Urban light pollution alters the diel vertical migration of *Daphnia*. Internationale Vereinigung fur Theoretische und Angewandte Limnologie Verhandlungen **27**: 779-782.

Muehlbauer, J. D., S. F. Collins, M. W. Doyle, and K. Tockner. 2014. How wide is a stream? Spatial extent of the potential “stream signature” in terrestrial food webs using meta-analysis. Ecology **95**: 44-55.

Nakano, S., and M. Murakami. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Sciences **98**: 166-170.

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

Nightingale, B., T. Longcore, and C. A. Simenstad. 2006. Artificial night lighting and fishes. Pages 257-276 *in* Rich C. and Longcore T. editors. Ecological consequences of artificial night lighting. Island Press, Washington DC, USA.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2013. Package ‘vegan’. Community ecology package, version 2. See https://cran.r-project.org/web/packages/vegan/ vegan.pdf.

Pawson, S., and M.-F. Bader. 2014. LED lighting increases the ecological impact of light pollution irrespective of color temperature. Ecological Applications **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. Freshwater Biology **59**: 368-377.

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

Péry, A. R., R. Mons, P. Flammarion, L. Lagadic, and J. Garric. 2002. A modeling approach to link food availability, growth, emergence, and reproduction for the midge Chironomus riparius. Environmental Toxicology and Chemistry **21**: 2507-2513.

Pinheiro, J., D. Bates, S. DebRoy, and D. Sarkar. 2015. R Development Core Team. 2014. nlme: linear and nonlinear mixed effects models. R package version 3.1-117. See https://cran.r-project.org/web/packages/nlme/nlme.pdf.

Pinheiro, J. C., and D. M. Bates. 1995. Approximations to the log-likelihood function in the nonlinear mixed-effects model. Journal of Computational and Graphical Statistics **4**: 12-35.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**: 289-316.

Punzo, F., C. Farmer, and J. Cook. 2006. Life history and ecology of the wolf spider *Pardosa sierra* Banks (Araneae: Lycosidae) in southeastern Arizona. The Southwestern Naturalist **51**: 310-319.

R Core Team 2015. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2013. Document freely available on the internet at: http://www. r-project. org.

Richardson, J. S., Y. Zhang, and L. B. Marczak. 2010. Resource subsidies across the land–freshwater interface and responses in recipient communities. River Research and Applications **26**: 55-66.

Schroer, S., and F. Hölker. 2016. Impact of lighting on flora and fauna. Pages 1-33 *in* Karlicek et al. editors. Handbook of Advanced Lighting Technology Reference. Springer International Publishing.

Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality (complete samples). Biometrika **52**: 591-611.

Spoelstra, K., R. H. A. van Grunsven, M. Donners, P. Gienapp, M. E. Huigens, R. Slaterus, F. Berendse, M. E. Visser, and E. Veenendaal. 2015. Experimental illumination of natural habitat—an experimental set-up to assess the direct and indirect ecological consequences of artificial light of different spectral composition. Philosophical Transactions of the Royal Society of London B: Biological Sciences **370**, 20140129.

Stewart-Oaten, A., W. W. Murdoch, and K. R. Parker. 1986. Environmental impact assessment:" Pseudoreplication" in time? Ecology **67**: 929-940.

Tabor, R. A., G. S. Brown, and V. T. Luiting. 2004. The effect of light intensity on sockeye salmon fry migratory behavior and predation by cottids in the Cedar River, Washington. North American Journal of Fisheries Management **24**: 128-145.

Takimoto, G., T. Iwata, and M. Murakami. 2002. Seasonal subsidy stabilizes food web dynamics: balance in a heterogeneous landscape. Ecological Research **17**: 433-439.

van Geffen, K. G., R. H. A. Grunsven, J. Ruijven, F. Berendse, and E. M. Veenendaal. 2014. Artificial light at night causes diapause inhibition and sex-specific life history changes in a moth. Ecology and Evolution **4**: 2082-2089.

van Grunsven, R. H. A., M. Donners, K. Boekee, I. Tichelaar, K. van Geffen, D. Groenendijk, F. Berendse, and E. Veenendaal. 2014. Spectral composition of light sources and insect phototaxis, with an evaluation of existing spectral response models. Journal of Insect Conservation **18**: 225-231.

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. Biological Conservation **144**: 2274-2281.

Waite, T. A., and L. G. Campbell. 2006. Controlling the false discovery rate and increasing statistical power in ecological studies. Ecoscience **13**: 439-442.

Wallace, J. B., S. Eggert, J. L. Meyer, and J. Webster. 1999. Effects of resource limitation on a detrital-based ecosystem. Ecological Monographs **69**:409-442.

Ward, J. V., and J. Stanford. 1982. Thermal responses in the evolutionary ecology of aquatic insects. Annual Review of Entomology **27**: 97-117.

Wei, C.-L., G. T. Rowe, R. L. Haedrich, and G. S. Boland. 2012. Long-term observations of epibenthic fish zonation in the deep Northern Gulf of Mexico. PloS One **7**: 1-14.

Wilk, M. B., and R. Gnanadesikan. 1968. Probability plotting methods for the analysis for the analysis of data. Biometrika **55**: 1-17.

4. Dietary changes in predators and scavengers in a riparian ecosystem illuminated at night

Manfrin A.[1, 2, 3], Lehmann D.[4, 5], van Grunsven R. H. A.[1], Larsen S.[6], Syväranta J.[7],
Wharton G.[3], Voigt C. C.[4], Monaghan M. T.[1*], Hölker F.[1*]

[1] Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

[2]Department of Biology-Chemistry-Pharmacy, Freie Universität Berlin, Berlin, Germany

[3]School of Geography, Queen Mary University of London, London, England, UK

[4]Leibniz-Institute for Zoo and Wildlife Research, Berlin; Germany

[5]Department of Biological and Environmental Sciences, University of Stirling, Stirling UK

[6]German Centre for Integrative Biodiversity Research, Leipzig, Germany

[7]Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland

*contributed equally

4.1 Abstract

Riparian habitats are characterized by fluxes of energy and matter between aquatic and terrestrial ecosystems forming subsidies for the recipient ecosystem. Artificial light at night (ALAN) from streetlamps is particularly widespread along waterways and can increase the flux of aquatic insects into terrestrial ecosystems and change the composition of terrestrial arthropod communities. Here we used stable carbon isotope analysis to test whether an increased abundance of aquatic prey in an ALAN-lit area resulted in a change in the diet of terrestrial arthropod consumers in an experimentally lit agricultural drainage ditch system in northern Germany.

The carbon isotopic signature of *Pachygnatha clercki* (Tetragnathidae) was 0.7‰ lower in lit traps compared to control traps in summer, indicating a greater assimilation of aquatic prey when the large majority of adult insects at lights were aquatic in origin. Bayesian mixing models also showed a 13% increase in aquatic prey intake in summer. In spring, isotopic signatures were more similar to terrestrial prey in lit traps compared to dark traps for *P. clercki* (0.3‰) and *Pardosa prativaga* (0.7‰), despite 80% of prey being aquatic at both sites. Bayesian mixing models showed increased terrestrial prey intake in all three taxa analysed (*P. clercki* and Opiliones 4%, *P. prativaga* 9%). In autumn, mixing models also indicated greater assimilation of terrestrial carbon for *P. prativaga* (5%) and Opiliones (7%) in lit traps despite there being a higher proportion of aquatic insects at the lit site.

Artificial illumination of the ecosystem changed the dietary composition of riparian predatory and scavenging invertebrates by altering the flux of aquatic insects. These changes were species-specific and varied among seasons, causing changes in terrestrial community composition and functioning. The large number of streetlights that occur near freshwaters worldwide can therefore have a large effect on aquatic-terrestrial ecosystem functioning.

4.2 Introduction

Complex trophic connections among organisms can extend across ecosystem boundaries (Polis et al. 1997). This is particularly evident in riparian zones where fluxes of nutrients and organic matter link adjacent aquatic and terrestrial ecosystems (Baxter et al. 2005). Such fluxes can cause a strong bottom-up effect for consumers in receiving habitats as resource subsidies (Polis et al. 1997, Nakano and Murakami 2001, Richardson et al. 2010). Quantifying these resource exchanges and measuring their effects on consumers is crucial for understanding the strength and the direction of the interaction of such coupled ecosystems (Marczak et al. 2007, Hoekman et al. 2011). Stable isotope methods have been used to quantify these fluxes and to better understand food webs that cross aquatic-terrestrial ecosystem boundaries. The stable isotope signal of carbon differs between aquatic and terrestrial primary producers because of the difference in the uptake of CO_2 in water and air (Rounick and Winterbourn 1986, Peterson and Fry 1987). Organisms that consume different proportions of aquatic- and terrestrial-derived sources exhibit contrasting $\delta^{13}C$ values (e.g. Kato et al. 2004).

Stable isotope studies have revealed that many riparian consumers rely on aquatic subsidies in the form of emergent insects (Baxter et al. 2005). The overall contribution of aquatic carbon to riparian consumer biomass varies among habitats, seasons, and consumer taxa (Paetzold et al. 2005). Aquatic-derived sources can constitute up to 50% of the carbon in the diet of Tetragnathidae orb-weaver spiders inhabiting riparian canopies (Kelly et al. 2015) and temperate forests (Krell et al. 2015). This can reach 100% for individuals inhabiting meadows along riparian areas in temperate regions (Krell et al. 2015) and in desert streams (Sanzone et al. 2003). Wandering ground-dwelling predators like Lycosidae and Carabidae also consume locally abundant aquatic prey, and studies have reported that aquatic prey can constitute 15% of consumer body carbon in forests and up to 50-60% in vineyards and deserts (Collier et al. 2002, Sanzone et al. 2003, Paetzold et al. 2005, Krell et al. 2015).

Both abiotic and biotic factors can influence spatial and temporal variation in the availability and use of aquatic subsidies in riparian zones, (Sabo and Power 2002, Paetzold et al. 2005). The importance of aquatic subsidies generally decreases with distance from the stream edge. A recent review found the density of aquatic insects to be reduced by 50% after only 1.5 m from the water’s edge, with a small portion of this subsidy (10%) moving much further away (>500 m) (Muehlbauer et al. 2014). Seasonal variation in aquatic insect emergence results in varying contributions of aquatic subsidies to riparian spiders (Nakano and Murakami 2001, Kato et al. 2004, Paetzold et al. 2005). The natural dynamic of aquatic subsidies between aquatic and terrestrial ecosystems is also influenced by anthropogenic environmental changes (Schulz et al. 2015, Larsen et al. 2015). Increased water temperature can cause earlier reproduction and emergence in aquatic insects with faster larval development (Harper and Peckarsky 2006), thereby affecting the timing of aquatic subsidy availability in riparian areas. Faster larval development and smaller adult body size was observed in intermittent streams (Shama and Robinson 2006, Jannot et al. 2008, Mikolajewsky et al. 2015). Removal of the natural riparian vegetation can decrease inland dispersal and flight activity of aquatic insects (Petersen et al. 1999). Gergs et al. (2014) also found that the introduction of the invasive amphipod *Dikerogammarus villosus* reduced emergence of chironomids. These and other anthropogenic alterations can thus strongly alter the quantity, quality and timing of aquatic subsidy fluxes with consequences for recipient communities (reviewed in Schulz et al. 2015, Larsen et al. 2016).

Artificial light at night (ALAN) is a globally pervasive alteration of the landscape (Hölker et al. 2010, Falchi et al. 2016) that is particularly widespread near freshwaters (e.g., streams, lakes), where human populations are often concentrated (Kummu et al. 2011). The effect of ALAN on these ecosystems can be substantial, in particular on the aquatic insects that live as larvae in the water and then emerge as flying adults. ALAN has been found to decrease mean body size and taxonomic richness (family-level) in emerging aquatic insects (Meyer and Sullivan 2013). ALAN also attracts post-emerging aquatic insects into adjacent riparian ecosystems, thereby disrupting their natural dispersal patterns (Capter 3, Perkin et al. 2014, Horvath et al. 2009, Meyer and Sullivan 2013). In some cases, ALAN has been found to increase aquatic insect mortality by exhaustion or increased predation (Eisenbeis 2006, Horvath et al. 2009). All of these have the potential to significantly alter the energy flows between aquatic and terrestrial ecosystems.

Manfrin et al. (Chapter 3) found a 3-fold increase in aquatic insect emergence directly under streetlamps in July and an increase in the number of aquatic flying insects (mostly Ephemeroptera) attracted by ALAN in the riparian areas (seasonally varying from 10-fold in September to 460-fold in July). However terrestrial flying insects were also attracted by ALAN (from 3-fold in October to 69-fold in July); the proportion of insects of aquatic origin caught in the lit traps was between 1.3-fold in September, 1.6-fold in July and 4.7-fold in October higher than in the control traps. We also observed simultaneous changes in the riparian community of spiders and scavengers, with significant changes in the abundance of thick-jawed spiders *Pachygnata clercki* (Tetragnatidae), wolf spiders *Pardosa prativaga* (Lycosidae), and harvestmen (Opiliones) in ALAN-exposed areas in one or more seasons.

Here we address the question of whether the large increase in input of freshwater-derived prey in the riparian area caused by ALAN led to a change in the relative consumption of aquatic and terrestrial prey by riparian consumers. We used Bayesian mixing models of $\delta^{13}C$ values to quantitatively infer the relative contribution of aquatic (e.g. non-biting midges, mayflies) and terrestrial prey (e.g. aphids, leaf hoppers) to the consumer diet under natural (control) and altered (treatment) light regimes across three seasons in 2013. We tested the hypothesis that the increased abundance of aquatic insects in the ALAN-treated area in the summer months would lead to an increase in aquatic stable isotope signal in the riparian community of spiders and scavengers.

4.3 Methods

4.3.1 Study area

The field experiment was carried out using a large-scale experimental infrastructure fully described by Holzhauer et al. (2015) (see also Chapter 3). It is located in the Westhavelland Nature Park and within a 750-km² International Dark-Sky Reserve that is one of the least illuminated areas in Germany (International Dark Sky Association, IDA 2015). In April 2012, two managed grassland areas with no prior exposure to ALAN were selected for an experiment to study the impact of artificial light on aquatic and terrestrial ecosystems. The two sites were environmentally similar in other prospectives than artificial light (see Chapter 3). Monitoring started at the beginning of May 2012, prior to any illumination. From July 25 onward, one site (the treatment site) was illuminated at night by three parallel rows of four streetlights located 20 m apart (see Fig. 1 in Chapter 3). Each streetlight was five meters high and equipped with one 70-W high-pressure sodium lamp (OSRAM VIALOX NAV-T Super 4Y). Both sites are adjacent to an agricultural drainage ditch and the parallel rows of streetlights were located 3 m, 23 m, and 43 m away from the water (see Fig. 1 in Chapter 3). During the course of the experiment, one set of streetlights was illuminated and the other set was not thus providing a control (dark) site. During the period of illumination, the treatment site was lit between civil twilight at dusk and dawn (see Holzhauer et al. 2015 for further details).

4.3.2 Study species

We studied five consumer species that, of the 42 predator and scavenger taxa examined in our previous study (Chapter 3), contributed significantly to changes in community composition through their shifts in abundance in lit traps. Two species were spiders, *Pachygnatha clercki* (Tetragnathidae) and *Pardosa prativaga* (Lycosidae) and three species were long-legged harvestmen (Opiliones). Opiliones species composition varied seasonally, thus we studied *Rilaena triangularis* in spring, *Nelima sempronii* and *Phalangium opilio* in summer and *N. sempronii* in autumn. For statistical analyses (see below), data from these three species of Opiliones were combined. Adults of *P. clercki* are night-active visual hunters and do not use webs (Keer et al. 1989). This is an atypical feeding strategy for Tetragnathidae, as most species build webs and are sit-and-wait predators. In our previous study, *P. clercki* was more abundant in lit traps at night in all seasons and extended its activity into the day in summer. *P. prativaga* is a vagrant day-active spider that catches prey without using a web (Kuusk and Ekbom 2010). This species was more abundant in lit traps during the day in summer and autumn, but was less abundant during the day in lit traps in spring. Opiliones are mainly active at night (Williams 1962) and in our experiment, were almost exclusively caught at night. Opiliones either ambush live prey or feed on dead animals. They do not employ webs (Pinto-da-Rocha et al. 2007). In our study site, they were more abundant in lit traps in spring and summer (Chapter 3).

4.3.3 Sample collection

Emerging adult aquatic insects (e.g. mayflies, caddisflies, non-biting midges) were collected using four emergence traps, one placed on the water surface in front of each street light (see Fig. 1 in Chapter 3). Insect sampling occurred monthly from May to

October 2013 except in July when sampling occurred weekly because emergence rates were very high. At each time, sampling was continuous for 128 - 192 hours. Aquatic and terrestrial flying insects (e.g. mayflies, moths, flying ground beetles) were collected using air eclector traps consisting of two transparent plexiglas panels. Traps were placed 0.5 m below each lamp (see Fig. 1 in Chapter 3). Ground-dwelling arthropods were collected using 24 pitfall traps per site, positioned between and under the streetlamps at different distance from the ditch (see Fig. 1 in Chapter 3). Air eclector and pitfall trap sampling occurred bi-weekly from May to October 2013. Sampling always occurred on rainless nights within one night of each half-moon phase (first and third quarter, s. a. Holzhauer et al. 2015). All samples were stored in 70% ethanol (Sarakinos et al. 2002).

4.3.4 Stable isotope analysis

We analysed up to 20 individuals of each consumer (*Pachygnatha clercki*, *Pardosa prativaga,* Opiliones) and of each potential prey taxon (10 taxa were considered potential prey; see Appendix S12) at each site (control, treatment) and season (spring, summer, autumn). Potential prey were selected based on evidence that they contribute to the diet of the studied consumers (Nyffeler and Benz 1988, Pinto-da-Rocha et al. 2007). A total of 294 consumer individuals (*P. clercki.,* n = 116*; P. prativaga,* n = 120*;* and Opiliones, n = 57) and 544 prey individuals (aquatic, n = 165; terrestrial, n = 379) were washed with distilled water in the laboratory, oven-dried at 70°C for 4 days, and ground to a fine powder using a milling machine (Pulveristette 23; Fritsch GmbH, Germany). An aliquot of each sample (0.5 - 2 mg) was weighed on a microbalance (Sartorius, Germany) and loaded into tin capsules (Costech Analytical Technologies, Valencia, CA) for stable isotope analysis. Lipids were not extracted from the samples. A preliminary comparison performed on five different taxa (see Appendix S10) found no difference between stable isotope values of fat- extracted and control samples (t-test, $p > 0.05$) (see Appendix S10 for fat extraction methodology).

We used an elemental analyser (Flash EA; Thermo Finnigan, Bremen, Germany) connected via a continuous flow system to an isotope ratio mass spectrometer (Delta V Advantage, Thermo Finnigan, Bremen, Germany) that measures the $\delta^{13}C$ and $\delta^{15}N$ of CO_2 and N_2 gases obtained after sample combustion. The sample isotope ratios were compared with international standards (USGS-24 and

IAEA N1) (Gonfiantini et al. 1995, Voigt et al. 2003). δ notation is used to express sample isotope ratios as parts per thousand (‰) differences to international standards of Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen (Slater et al. 2001). The instrument precision was better than 0.1‰ (one standard deviation).

4.3.5 Statistical analysis

We performed statistical analysis using only $\delta^{13}C$ values because these provided a distinct separation between aquatic and terrestrial prey throughout the experiment (see Results). The $\delta^{15}N$ values were highly variable and showed no clear differentiation between aquatic and terrestrial sources or among trophic levels (Appendix S11). They were therefore not used in the statistical analyses. Because our aim was to test whether consumer diet shifted as a result of changing relative abundances of aquatic and terrestrial prey, prey taxa were pooled and classified as aquatic or terrestrial in origin. Pooling multiple source species into biologically meaningful groups is the recommended practise when within-group isotopic variation is smaller than between-group variation, providing more constrained and less diffuse solutions of models using isotope values (Phillips et al. 2005, Phillips et al. 2014). We also corrected consumer $\delta^{13}C$ for trophic fractionation by 1‰ (DeNiro and Epstein 1978, Akamatsu, et al. 2004).

Differences in prey and consumer $\delta^{13}C$ were analysed with linear mixed-effect (LME) models using the lme4 package (Bates et al. 2007) for R (R Core Team 2015). Fixed factors for the prey model were “habitat” (aquatic or terrestrial), “site” (control or treatment), “season” (spring: May – June; summer: July – August; autumn: September – October) and their interactions. Fixed factors for the consumer model were “taxa” (i.e. *P. clercki*, *P. prativaga*, Opiliones), “site”, “season” and their interactions. As post-hoc pairwise comparison, another LME model was used for each of the three consumer taxa within each season, in which “site” was a unique fixed factor. All LME models considered “trap” nested in “site” as random factors to account for multiple observations. Each LME model was compared with a reduced model (i.e. without the fixed factors) using a likelihood ratio test (Pinheiro and Bates 1995). The distribution of residuals was assessed using Wilk-Shapiro tests (Shapiro and Wilk 1965) and qq-plots (Wilk and Gnanadesikan 1968). To control for inflated false discovery rates, we used Benjamini-Hochberg corrected α-values (Waite and Campbell 2006).

In addition to LME models testing for significance, we used model-based estimates of the relative contribution of aquatic and terrestrial food sources to consumer diets using the mixing model package SIAR (Parnell and Jackson 2013) for R. SIAR uses Bayesian inference to calculate the most likely set of dietary proportional contributions given the isotopic ratios in a set of possible food sources and consumers (Parnell et al. 2010). This generates potential dietary solutions as Dirichlet probability distributions with mean, mode, and levels of uncertainty (95% credibility intervals). We ran 1 million iterations, thinned by 300 and with an initial discard of the first 40,000 iterations. Control and treatment sites were compared across the three seasons in 2013.

4.4 Results

$\delta^{13}C$ was significantly lower in aquatic prey (-34.0 ± 2.2‰) compared to terrestrial prey (-26.5 ± 1.24‰) (Table 1, Fig. 1, Appendix S12). There were no significant differences in prey $\delta^{13}C$ mean values between control and lit sites (Fig. 1, Table 1). In each consumer taxon, mean $\delta^{13}C$ was more similar to that of terrestrial prey than aquatic prey in both control and lit sites (Fig. 2, Appendix S12). Nonetheless, consumer $\delta^{13}C$ values varied between sites and among seasons (Table 1). The effect of treatment differed across taxa (site x taxa interaction, Table 1, Fig. 2) and among seasons (site x season interaction, Table 1, Fig. 2). In *Pachygnatha clercki*, $\delta^{13}C$ was 0.3‰ higher at the lit site than at the control site in spring ($F_{1, 40} = 5.4$; $p = 0.02$) but was 0.7‰ lower at the lit site in summer ($F_{1, 36} = 8.20$; $p <0.001$) (Fig. 2). In *Pardosa prativaga,* $\delta^{13}C$ was 0.7‰ higher at the lit site in spring ($F_{1,40} = 16.7$; $p < 0.001$) and 0.5‰ higher at the lit site in summer ($F_{1,40} = 9.4$; $p = 0.003$) (Fig. 2). In Opiliones, there were no differences among sites and seasons (Fig. 2).

Bayesian mixing models (SIAR) indicated that the contribution of terrestrial-derived carbon to consumer diet in the control site ranged from 67-80% (aquatic-derived carbon ranged from 20-32%), with variation occurring among taxa and seasons (see Fig.3, Appendix S13). In summer, *P. clercki* at the lit site exhibited a 13% increase in aquatic prey intake compared to the control site, whereas the contribution of aquatic prey to the diet of *P. prativaga* and Opiliones (*N. sempronii* and *P. opilio* in summer*)* was similar at both sites (Fig. 3, Appendix S13). In spring, Bayesian mixing models showed increased terrestrial prey intake at the lit site in *P.*

clercki and Opiliones (4%) and in *P. prativaga* (9%) (Fig. 3, Appendix S13). In autumn, mixing models also indicated greater assimilation of terrestrial carbon at the lit site for *P. prativaga* (5%) and Opiliones (7%) (Fig. 3, Appendix S13).

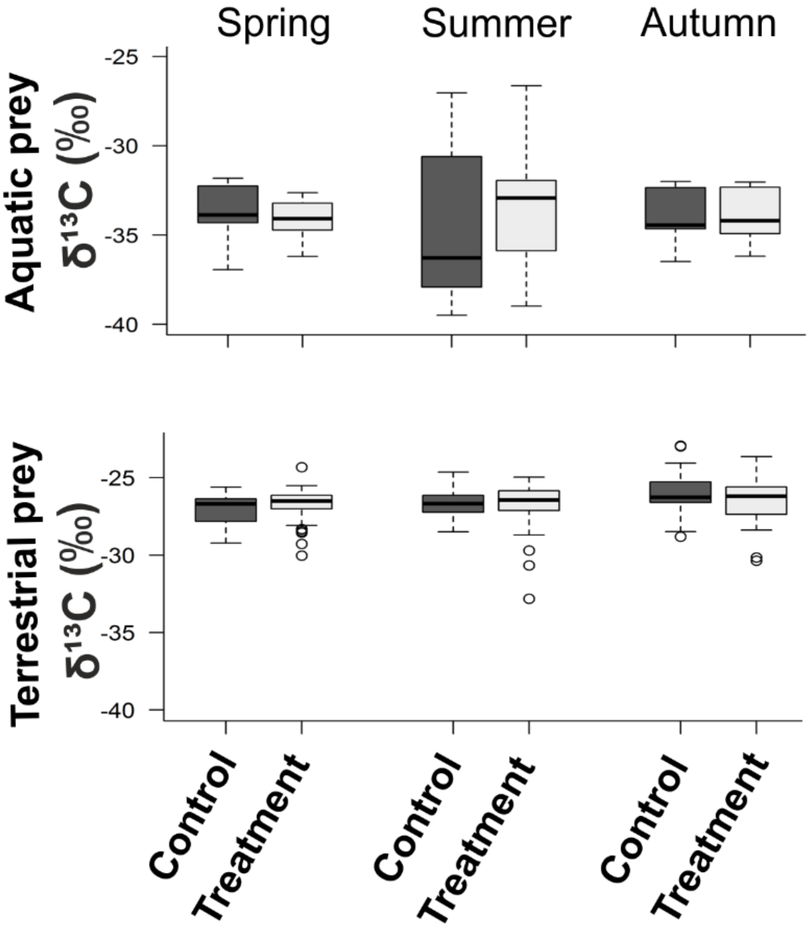


Figure 1. Comparison of $\delta^{13}C$ values between control and treatment sites is depicted for aquatic and terrestrial prey over the three seasons in 2013. Box plots depict the 25, 50 and 75 percentiles, and whiskers the highest and lowest values excluding outliers.

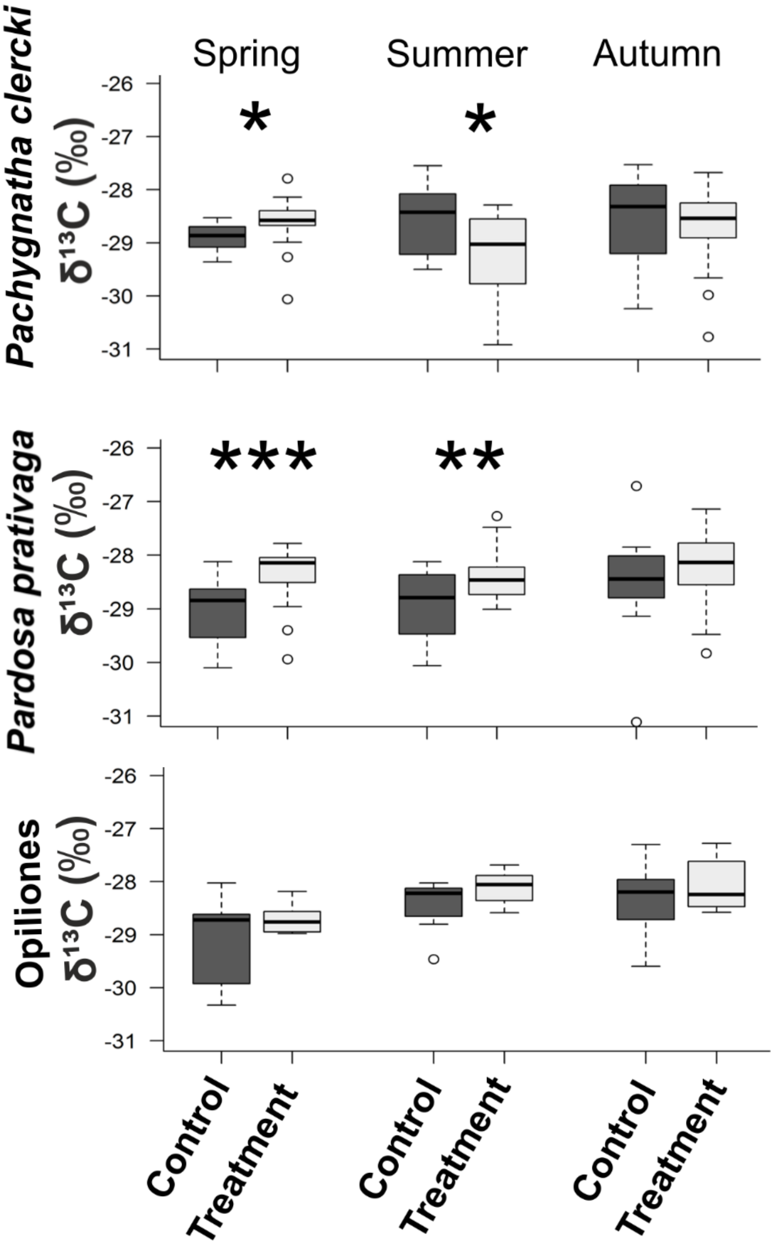


Figure 2. Comparison of $\delta^{13}C$ values between control and treatment sites is depicted for consumer taxa over the three seasons in 2013. Box plots depict the 25, 50 and 75 percentiles, and whiskers the highest and lowest values excluding outliers. In case of significant LME interaction, asterisks are used to indicate significant difference in the pairwise comparisons (*** = p<0.001; ** = p<0.01; * = p<0.05).

Table 1. Results of linear mixed-effect models (LME) for prey and consumers using $\delta^{13}C$ as a dependent variable. Independent variables (Factors) for food sources and consumers are shown in the table. Asterisks are used to indicate significant main effect (*** = p<0.001; ** = p<0.01; * = p<0.05).

Model	X^2	Factors	*F*-statistic
Prey	346.56***	Site	$F_{1,91}$=0.10
		Habitat	$F_{1,27}$=8.55***
		Season	$F_{2,520}$=4.13*
		Site x Habitat	$F_{1,91}$=0.18
		Site x Season	$F_{2,520}$=1.37
		Habitat x Season	$F_{2,520}$=0.62
		Site x Habitat x Season	$F_{2,420}$=2.74
Consumers	62.73***	Site	$F_{4,293}$=8.53**
		Taxa	$F_{1,293}$=4.78**
		Season	$F_{2,293}$=8.71***
		Site x Taxa	$F_{2,293}$=8.59***
		Site x Season	$F_{2,293}$=2.33*
		Taxa x Season	$F_{4,293}$=2.74*
		Site x Taxa x Season	$F_{4,293}$=1.70

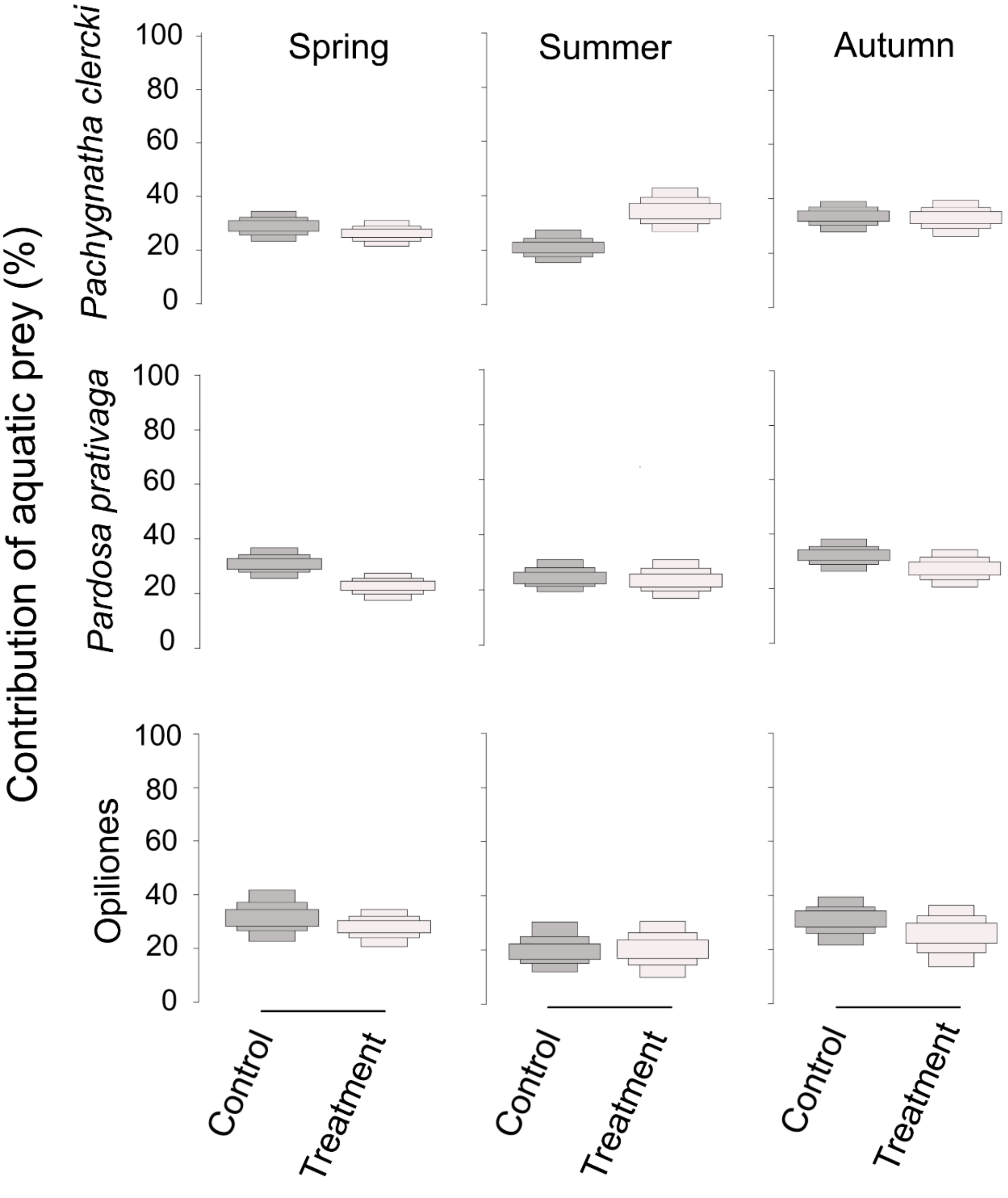


Figure 3. Comparison of the relative contribution (%) of aquatic prey to the diets of the consumer species based on Bayesian isotope mixing models on $\delta^{13}C$ values. The plots show 95% (middle rectangle), 75% and 25% (external rectangles) credibility intervals. Results are shown for control and treatment site across the three seasons in 2013.

4.5 Discussion

The importance of aquatic subsidies as a food source for riparian arthropods is well documented (e.g. Collier et al 2002, Sabo and Power 2002, Sanzone et al. 2003, Kato et al. 2003, Paetzold et al. 2011). Here we tested whether ALAN, which several studies have found increases the local abundance of adult aquatic insects as potential prey (Perkin et al. 2014, Horvath et al. 2009) (Chapter 3), caused a shift in the diet of riparian arthropod predators and scavengers. We used stable isotopes to examine the proportion of aquatic and terrestrial carbon sources at control and lit traps in three seasons.

Terrestrial and aquatic prey species differed in $\delta^{13}C$, allowing us to differentiate these two source categories in the diet of our consumers. This was expected, as carbon isotope signatures can often distinguish between aquatic and terrestrial ecosystems (Kato et al. 2004). We observed no direct effect of ALAN on $\delta^{13}C$ values of either aquatic or terrestrial prey, suggesting that ALAN did not affect their isotopic composition. We therefore conclude that changes in $\delta^{13}C$ observed in the consumers in the treatment site resulted from changes in prey consumption.

The proportion of aquatic prey (20 - 33%) in the diet of the riparian consumers at the control site indicates that aquatic insects were an important food source for them. These proportions are comparable to those observed in riparian canopies and forests in northern temperate regions (Briers et al. 2005, Krell et al. 2015) but lower than those observed in riparian areas of desert streams (Sanzone et al. 2003). The degree to which consumers respond to aquatic subsidies depends on the ratio of aquatic to terrestrial resources in the recipient habitat (Marczak et al 2007). This ratio can strongly differ among habitats. For instance, stronger gradients in productivity exist between aquatic and riparian areas in desert zones compared to temperate zones, with temperate riparian areas generally being more productive. In desert zones, the aquatic insect contribution for active-hunting spiders (i.e. not using webs) can reach 70% (Sanzone et al. 2003) while in temperate zones, as in our case, aquatic insects can contribute from 15 to 50% (Briers et al. 2005, Krell et al. 2015). In our control site, the spring and autumn values for the proportion of aquatic prey (30-33%) were higher than in summer (20-24%). This pattern indicates a seasonal change in consumer diet that may be explained by the seasonal availability of aquatic (emerging and flying) and terrestrial prey (flying and ground-dwelling) caught during the experiment at the control site (Chapter 3). The relation found between prey availability and prey consumption from generalist predators is also in line with what has been found in other studies (Kato et al. 2004, Paetzold et al. 2005, 2006). A seasonal pulse of aquatic subsidies is particularly common at northern temperate latitudes. In such regions, water temperature and photoperiod play an important role in regulating aquatic insect emergence and are seasonally variable (Corbet 1964, Brittain 1982, Paezold et al. 2005).

When exposed to ALAN in our study, *Pachygnatha clercki* increased its assimilation of aquatic-derived carbon in summer according to both the isotopic signature and the Bayesian model analysis of the diet. This shift in the spider diet was probably due to the large number of aquatic insects attracted to the light sources during the summer season. The number of aquatic insects caught at the treatment site at this time was approximately 25 times higher than in spring and 130 times higher than in autumn, and 87% of all collected insects (8000 individuals) were aquatic, compared to 15% (140 individuals) caught at the control site (Chapter 3) (see appendix S14). *P. clercki* is primarily a night-active spider, but extended its activity into the day when exposed to ALAN in summer (Chapter 3). It may be that *P. clercki* consumed exhausted or dead aquatic insects lying on the ground after flying around the lamps during the night. Although spiders rarely feed on dead prey, von Berg et al. (2012) found that 38% of the specimens of *Pachygnatha degeeri* analysed opportunistically scavenged when dead prey was available.

The increased assimilation of aquatic-derived carbon found in *P. clercki* in summer was not observed in *P. prativaga* or Opiliones. These taxa might simply have maintained their preference for larger-sized terrestrial prey over the numerous but smaller aquatic prey (Briers et al. 2005). In contrast to *P. clercki*, *P. prativaga* and Opiliones did not exploit the additional extra hours of hunting-activity during the day (Chapter 3), and therefore may not have utilized dead insects on the ground from the night before. Alternatively, the carbon derived from aquatic prey in summer, might not have been integrated into consumer tissues. These taxa may have allocated most of the food intake in this period to reproduction as metabolic carbon instead of structural carbon for somatic growth (Jespersen and Toft 2003, Bragg and Holmberg 2009). The isotopic signatures and analysis of the diet composition suggest an increased terrestrial prey intake by *P. clercki* in spring and in *P. prativaga* and Opiliones (*R. triangularis* and *N. sempronii*) in spring and autumn when exposed to ALAN. The aquatic insects collected in our traps (predominantly Chironomidae and *Cloeon* sp.) were smaller than many terrestrial taxa available as prey (e.g. leaf hoppers, moths). This difference in biomass might explain why consumers increased the terrestrial prey intake under artificial illumination, with an overall lower number of available prey (in both spring and autumn) than in summer or with more similar abundance between aquatic and terrestrial prey (in autumn) (Chapter 3). Thus, the effect of ALAN might strongly depend on the phenology of the sources subsidies and on both phenology and feeding strategies of the consumers.

Our results provide evidence that ALAN can change the dietary composition of secondary consumers. A shift to more reliance on an aquatic-derived diet can affect the flow of energy through the food-web. It is well known that spiders are important biological control agents (Riechert et al. 1984, Hodge 1999, Marc et al. 1999, Henschel et al. 2001). Dietary shifts observed under ALAN, arising from a disproportionate availability of a specific prey type (e.g. aquatic prey), might release predatory pressure from species causing a displacement of predator-prey dynamics. In the case of semi-urban and agricultural areas, this might have consequences for the natural control of invertebrate pest populations (e.g. Aphidae, Auchenorrhyncha) by predation (Dixon 2000, Hassell 1978, Polis and Strong 1996).

In considering the findings from this study, it is important to acknowledge that our experiment used standard streetlights and high-pressure sodium lamps. These lamps are considered to be relatively ‘insect friendly’ (Eisenbeis 2006). However, the increasing use of LED lamps, which have spectral emission peaks in white-blue, may have even more detrimental effects on nocturnal invertebrates given their sensitivity to short wavelength light (van Langevelde 2011, van Grunsven et al. 2014, Pawson and Bader 2014). This would have major implications for conservation biology, as well as for lighting policy and landscape planning. Furthermore, we found these results in an agricultural drainage ditch system with highly productive riparian areas, i.e. an abundance of terrestrial resources. Because the effects of donor subsidies on recipient ecosystems are usually stronger when the receiving system has low levels of resources (Marczak et al. 2007), the introduction of ALAN might have more substantial effects in riparian areas with stronger gradients in productivity between aquatic and riparian zones. Limiting exposure of streams to ALAN during periods where emergence peaks and when terrestrial arthropod activity is high might be a first measure to mitigate the propagation of the effect to the riparian consumer communities.

4.6 Acknowledgements

We thank Stefan Heller, Maja Grubisic, Sibylle Schroer, Liliana Lehmann, Nina-Sophie Weiss and Babette Pohlmann for help during the experiment and in the laboratory analysis. We thank Francesca Pilotto and Thomas Mehner for advice on statistical analysis. This work was 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 (FKZ 3514821700). S. Larsen was supported by an individual fellowship from the German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany. We acknowledge financial support from the Academy of Finland (grant 296918) to Jary Syväranta.

4.7 References

Akamatsu, F., H. Toda, and T. Okino. 2004. Food source of riparian spiders analyzed by using stable isotope ratios. Ecological Research **19**: 655-662.

Bates, D., D. Sarkar, M. D. Bates, and L. Matrix. 2007. The lme4 package. R package version 2.

Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology **50**: 201-220.

Bragg, P. D., and R. G. Holmberg. 2009. The Harvestmen (Arachnida, Opiliones) of British Columbia. Journal of the Entomological Society of British Columbia **106**: 29-37.

Briers, R. A., H. M. Cariss, R. Geoghegan, and J. H. Gee. 2005. The lateral extent of the subsidy from an upland stream to riparian lycosid spiders. Ecography **28**: 165-170.

Brittain, J. E. 1982. Biology of mayflies. Annual review of entomology **27**: 119-147.

Collier, K. J., S.Bury, and M. Gibbs. 2002. A stable isotope study of linkages between stream and terrestrial food webs through spider predation. Freshwater Biology **47**: 1651-1659.

Corbet, P. S. 1964. Temporal patterns of emergence in aquatic insects. The Canadian Entomologist **96**: 264-279.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta **42**: 495-506.

Dixon, A. F. G. 2000. Insect predator-prey dynamics: ladybird beetles and biological control. Cambridge University Press.

Eisenbeis, G., C. Rich, and T. Longcore, 2006. Artificial night lighting and insects: attraction of insects to streetlamps in a rural setting in Germany. Pages 191-198 *in* Rich, G. C. and Longcore, T. (eds). Ecological consequences of artificial night lighting. Island Press, Washington DC, USA.

Falchi, F., P. Cinzano, D. Duriscoe, C. C. Kyba, C. D. Elvidge, K. Baugh, B. A. Portnov, N. A. Rybnikova, and R. Furgoni. 2016. The new world atlas of artificial night sky brightness. Science Advances **2**: 1-25.

Gergs, R., M.Koester, R. S. Schulz, R. and Schulz. 2014. Potential alteration of cross-ecosystem resource subsidies by an invasive aquatic macroinvertebrate: implications for the terrestrial food web. Freshwater Biology **59**: 2645-2655.

Gonfiantini, R., W. Stichler, K. and Rozansky. 1995. Intercomparison materials for stable isotopes of light elements. International Atomic Energy Agency, Vienna, pp. 13.

Harper, M. P., and B. L. Peckarsky. 2006. Emergence cues of A mayfly in a high-altitude stream ecosystem: potential response to climate change. Ecological Applications **16**: 612-621.

Hassell, M. P. 1978. The dynamics of arthropod predator-prey systems. Princeton University Press.

Henschel, J. R., D. Mahsberg, and H. Stumpf. 2001. Allochthonous aquatic insects increase predation and decrease herbivory in river shore food webs. Oikos **93**: 429-438.

Hodge, M. A. 1999. The implications of intraguild predation for the role of spiders in biological control. Journal of Arachnology **27**: 351-362.

Hoekman, D., J. Dreyer, R. D. Jackson, P. A. Townsend, and C. Gratton. 2011. Lake to land subsidies: experimental addition of aquatic insects increases terrestrial arthropod densities. Ecology **92**: 2063-2072.

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

Holzhauer, S. I., S. Franke C. Kyba A. Manfrin, R. Klenke, C. C. Voigt, D. Lewanzik, M. Oehlert, M. T. Monaghan, S. Schneider S., Heller, H. Kuechly, A. Brüning, A. C. Honnen, and F. Hölker. 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.

Horváth, G., G. Kriska, P. Malik, and B. Robertson. 2009. Polarized light pollution: a new kind of ecological photopollution. Frontiers in Ecology and the Environment **7**: 317-325.

International Dark Sky Association (IDA). International Dark Sky Reserves: Westhavelland (Germany). Available online: http://darksky.org/ idsp/ reserves/ westhavelland/ (accessed on 13 November 2015).

Jannot. J. E., S. A. Wissinger, and J. R. Lucas. 2008. Diet and a developmental time constraint alter life-history trade-offs in a caddis fly (Trichoptera: Limnephilidae). Biological Journal of the Linnean Society **95**: 495-504.

Jespersen, L., and S. Toft. 2003. Compensatory growth following early nutritional stress in the wolf spider *Pardosa prativaga*. Functional Ecology **17**: 737-746.

Kato, C., T. Iwata, S. Nakano, and D. Kishi. 2003. Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. Oikos **103**: 113-120.

Kato, C., T. Iwata, and E. Wada. 2004. Prey use by web-building spiders: stable isotope analyses of trophic flow at a forest-stream ecotone. Ecological Research **19**: 633-643.

Keer, R. D., M. Alderweireldt, K. Decleer, H. Segers, K. Desender, and J. P. Maelfait. 1989. Horizontal distribution of the spider fauna of intensively grazed pastures under the influence of diurnal activity and grass height. Journal of Applied Entomology **107**: 455-473.

Kelly, S.P., E. Cuevas, and A. Ramírez. 2015. Stable isotope analyses of web-spinning spider assemblages along a headwater stream in Puerto Rico. PeerJ **3**, e1324.

Krell, B., N. Röder, M. Link, R. Gergs, M. H. Entling, and R. B Schäfer. 2015. Aquatic prey subsidies to riparian spiders in a stream with different land use types. Limnologica **51**: 1-7.

Kummu, M., H. De 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**:1-13.

Kuusk, A.-K., and B. Ekbom. 2010. Lycosid spiders and alternative food: feeding behavior and implications for biological control. Biological Control **55**: 20-26.

Larsen, S., J. D. Muehlbauer, and E. Marti. 2015. Resource subsidies between stream and terrestrial ecosystems under global change. Global Change Biology **22**: 2489-2504.

Marc, P., A. Canard, and F. Ysnel. 1999. Spiders (Araneae) useful for pest limitation and bioindication. Agriculture, Ecosystems and Environment **74**: 229-273.

Marczak, L. B., and J. S. Richardson. 2007. Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. Journal of Animal Ecology **76**: 687-694.

Mikolajewski D. J, M. De Block, and R. Stoks. 2015. The interplay of adult and larval time constraints shapes species differences in larval life history. Ecology **96**: 1128-1138.

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

Muehlbauer, J. D., S. F. Collins, M. W. Doyle, and K. Tockner. 2014. How wide is a stream? Spatial extent of the potential “stream signature” in terrestrial food webs using meta-analysis. Ecology **95**: 44-55.

Nakano, S., and M. Murakami. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Sciences **98**: 166-170.

Nyffeler, M., and G. Benz. 1988. Feeding ecology and predatory importance of wolf spiders (*Pardosa* spp.)(Araneae, Lycosidae) in winter wheat fields. Journal of Applied Entomology **106**: 123-134.

Paetzold, A., J. F. Bernet, and K. Tockner. 2006. Consumer-specific responses to riverine subsidy pulses in a riparian arthropod assemblage. Freshwater Biology **51**: 1103-1115.

Paetzold, A., C. J. Schubert, and K. Tockner. 2005. Aquatic terrestrial linkages along a braided-river: riparian arthropods feeding on aquatic insects. Ecosystems **8**: 748-759.

Paetzold, A., S. Marian, H. W. Philip, and L. Maltby. 2011. Environmental impact propagated by cross-system subsidy: Chronic stream pollution controls riparian spider populations. Ecology **92**: 1711-1716.

Parnell, A., and A. Jackson. 2013. SIAR: Stable isotope analysis in R. R package version 4.2. Available from: http://CRAN.R-project.org/package=siar.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. PloS One **5**, e9672.

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

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

Petersen, I., J. H. Winterbottom, S. Orton, N. Friberg, A. G. Hildrew, D. C. Spiers, and W. S. C Gurney. 1999. Emergence and lateral dispersal of adult Plecoptera and Trichoptera from Broadstone Stream, UK. Freshwater Biology **42**: 401-416.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics **18**: 293-320.

Phillips, D. L., R. Inger, S. Bearhop, A. L. Jackson, J. W. Moore, A. C. Parnell, B. X. Semmens, and E. J. Ward. 2014. Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology **92**: 823-835.

Phillips, D. L., S. D. Newsome, and J. W. Gregg. 2005. Combining sources in stable isotope mixing models: alternative methods. Oecologia **144**: 520–527.

Pinheiro, J. C., and D. M. Bates. 1995. Approximations to the log-likelihood function in the nonlinear mixed-effects model. Journal of Computational and Graphical Statistics **4**: 12-35.

Pinto-da-Rocha, R., G. Machado, and G. Giribet. 2007. Harvestmen: the biology of Opiliones. Harvard University Press.

Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. American Naturalist **147**: 813-846.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**: 289-316.

R Core Team 2015. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2013. Available on the internet at: http://www. r-project. org.

Richardson, J. S., Y. Zhang, and L. B. Marczak. 2010. Resource subsidies across the land–freshwater interface and responses in recipient communities. River Research and Applications **26**: 55-66.

Riechert, S. E., and T. Lockley1984. Spiders as biological control agents. Annual Review of Entomology **29**: 299-320.

Rounick, J., and M. Winterbourn 1986. Stable carbon isotopes and carbon flow in ecosystems. BioScience **36**: 171-177.

Sabo, J., and M. Power. 2002. River–watershed exchange: effects of riverine subsidies on riparian lizards and their terrestrial prey. Ecology **83**: 1860-1869.

Sanzone, D., J. Meyer, E. Martí, E. Gardiner, J. Tank, and N. Grimm. 2003. Carbon and nitrogen transfer from a desert stream to riparian predators. Oecologia **134**: 238-250.

Sarakinos, H. C., M. L. Johnson, and M. J. V. Zanden. 2002. A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. Canadian Journal of Zoology **80**: 381-387.

Schulz, R., M. Bundschuh, R. Gergs, C. A. Brühl, D. Diehl, M. H. Entling, L. Fahse, O. Frör, H. F. Jungkunst, A. Lorke, R. B. Schäfer, G. E. Schaumann, and K. Schwenk. 2015. Review on environmental alterations propagating from aquatic to terrestrial ecosystems. Science of the Total Environment **15**: 246–261.

Shama, L. N., and C. T. Robinson. 2006. Sex-specific life-history responses to seasonal time constraints in an alpine caddisfly. Evolutionary Ecology Research **8**: 169-180.

Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality (complete samples). Biometrika **52**: 591-611.

Slater, C., T. Preston, and L. T. Weaver. 2001. Stable isotopes and the international system of units. Rapid Communications in Mass Spectrometry **15**: 1270-1273.

van Grunsven, R. H. A., M. Donners, K. Boekee, I. Tichelaar, K. van Geffen, D. Groenendijk, F. Berendse, and E. Veenendaal. 2014. Spectral composition of light sources and insect phototaxis, with an evaluation of existing spectral response models. Journal of Insect Conservation **18**: 225-231.

van Langevelde, F., J. A. Ettema, M. Donners, M. F. Wallis DeVries, and D. Groenendijk. 2011. Effect of spectral composition of artificial light on the attraction of moths. Biological Conservation **144**: 2274-2281.

Voigt, C. C., F. Matt, R. Michener, and T. H. Kunz. 2003. Low turnover rates of carbon isotopes in tissues of two nectar-feeding bat species. Journal of Experimental Biology **206**: 1419-1427.

von Berg, K., M. Traugott, and S. Scheu. 2012. Scavenging and active predation in generalist predators: a mesocosm study employing DNA-based gut content analysis. Pedobiologia **55**: 1-5.

Waite, T. A., and L. G. Campbell. 2006. Controlling the false discovery rate and increasing statistical power in ecological studies. Ecoscience **13**: 439-442.

Wilk, M. B., and R. Gnanadesikan. 1968. Probability plotting methods for the analysis for the analysis of data. Biometrika **55**: 1-17.

Williams, G. 1962. Seasonal and diurnal activity of harvestmen (Phalangida) and spiders (Araneida) in contrasted habitats. The Journal of Animal Ecology **31**: 23-42.

5. General discussion

5.1 Rationale and thesis aims

Although inland waters cover less than 1% of the Earth's surface, they contain 10% of all known animal species. Over 60% of these species, or 100,000, are aquatic insects (Dijkstra et al. 2014) making preserving freshwater ecosystems, as biodiversity "hotspots", a priority. Freshwater ecosystems do not function in isolation (Schulz et al. 2015, Larsen et al. 2016). Aquatic and adjacent riparian habitats are coupled ecosystems whose dynamics and stability can only be understood in the context of such interdependence (Richardson et al. 2010, Muehlbauer et al. 2014, Gurnell et al. 2016, Larsen et al. 2016). Emerging aquatic insects create a cross-habitat linkage between donor-aquatic and recipient-terrestrial ecosystems and form an important subsidy to terrestrial consumers (Power et al. 2004, Gratton and Vander Zanden 2009, Larsen et al. 2016). Aquatic and riparian ecosystems are, in this way functionally linked; alterations in one ecosystem can affect the other triggering important functional impairments (Larsen et al. 2016).

Humans have extensively altered the physical, chemical, and biological features of aquatic and riparian habitats. The major drivers of this impact include human alteration of hydrogeomorphology, land-use, chemical and nutrient inputs and invasive species and frequently act simultaneously (Jackson et al. 2001, Carpenter et al. 2011, Tockner et al. 2010, Larsen et al. 2016). Although many biological patterns are regulated by natural light/dark cycles, changes of the natural dark environment due to ALAN are still rarely listed as an important threat to aquatic and riparian ecosystem biodiversity and functioning (Perkin et al. 2011). As ALAN has only recently been recognized as a worldwide human-induced impact, it is still poorly understood. Identifying the potential effects of ALAN on organisms and ecosystems is fundamental to protecting the future of such important natural systems.

The aims of this thesis were to investigate whether ALAN: affected macroinvertebrate density and community functional and taxonomical composition (Chapter 2); affected aquatic insect emergence, spatial and temporal distribution of flying aquatic and terrestrial insects in the riparian environment, and the abundance and composition of riparian ground-dwelling predator and scavenger communities

(Chapter 3); changes the diet composition of ground-dwelling secondary consumers in the riparian areas (Chapter 4).

5.2 Major findings and ecological implications

I found that ALAN can impact macroinvertebrate communities and that the effect of ALAN can propagate across ecosystem boundaries, altering subsidy fluxes for riparian ground-dwelling secondary consumers and affecting aquatic-riparian food web interactions.

In my first study, in which I experimentally introduced increased light levels to sub-alpine stream-side flumes, I found that ALAN increased the abundance of *Baetis* spp. and Chironomidae likely due to inhibition of drifting behaviour. This resulted in changes in taxonomical and functional composition of macroinvertebrate communities. According to Perkin et al. (2014b) that found a clear inhibition of the drifting activity in *Baetis* spp. in headwaters streams of coastal British Columbia, we can conclude that the inhibitory effect of ALAN on the mayfly *Baetis* spp. is strong and widespread in different stream typologies. The effect of ALAN on macroinvertebrate density and community composition was detected only during spring and not in autumn. In autumn, the density of macroinvertebrates in the flumes was 4 times higher than in spring. In this context, higher competition for resources or space than in spring due to higher density might have overridden the effect of ALAN by stimulating drift regardless of ALAN.

The effect of ALAN propagated across ecosystem boundaries affecting both the aquatic larval and the adult life stage of aquatic insects. In the agricultural ditch, ALAN increased the number of emerging aquatic insects and the proportion of aquatic flying insects (mainly *Cloeon* sp.) attracted in the riparian areas (Chapter 3) modifying natural aquatic-terrestrial subsidy dynamics. As aquatic subsidies form an important component of the diet of many riparian invertebrate consumers (Baxter et al. 2005), changes in such prey fluxes caused by ALAN were likely the main driver of the change in taxonomical (Chapter 3) and dietary (Chapter 4) composition (see Fig. 1) observed in the ground-dwelling secondary consumer community. The effect of ALAN on secondary consumers varied among taxa and seasons suggesting a strong dependency on e.g. the feeding behaviour or diel activity (day-, night-active) of the taxa. For example, night-active spiders (e.g. *Pachygnatha clercki*) and carrion beetles

(*Silpha obscura*) extended their activity into the day when exposed to ALAN likely feeding on exhausted or dead insects that were attracted to the lights the night before. Extension of the natural nocturnal activity of these ALAN-tolerant taxa might affect the ecosystem temporal niche partitioning increasing competition for resources (space, food) with unknown consequences for the functioning of the aquatic-terrestrial ecosystem linkage.

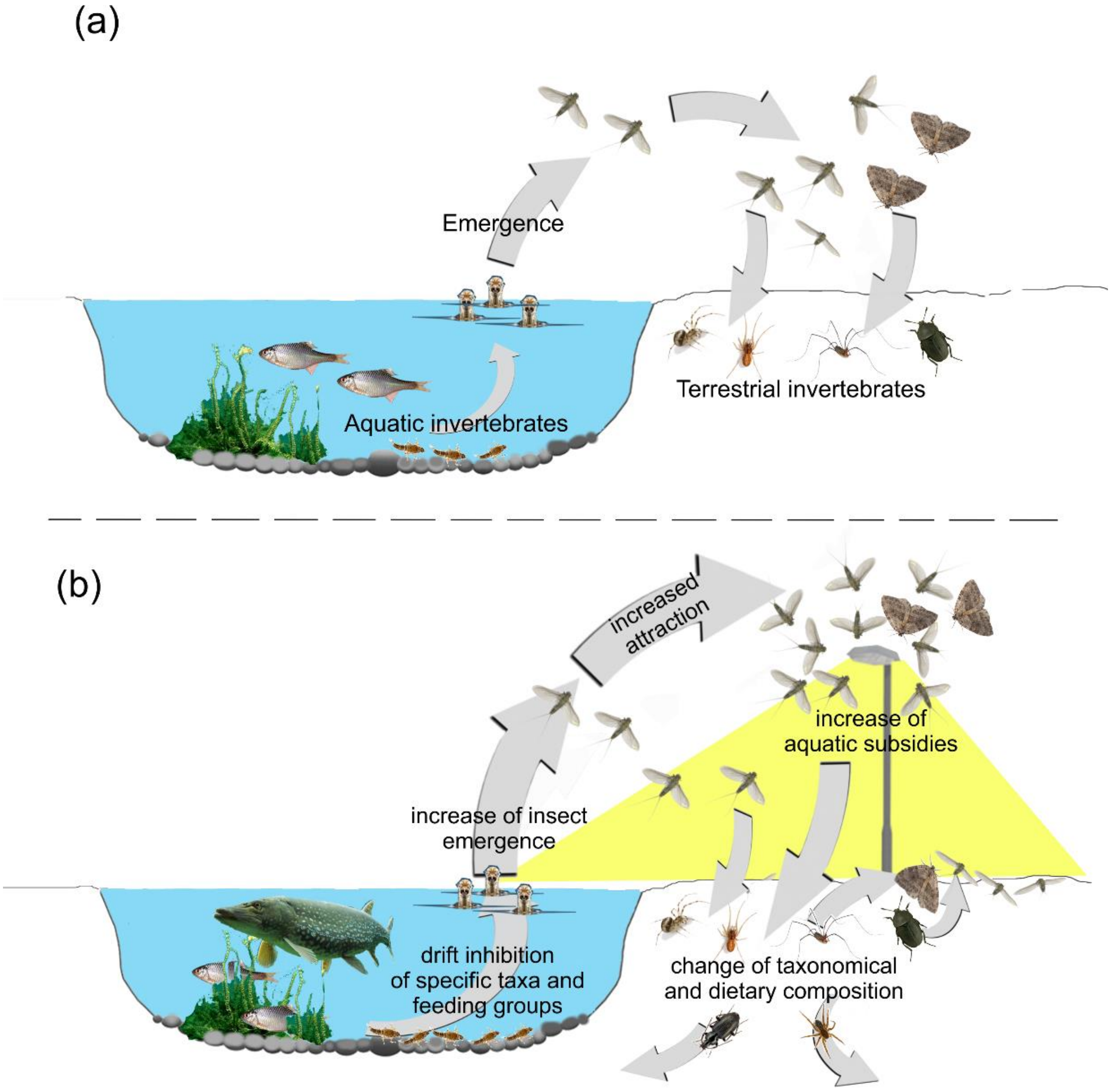


Figure 1. Aquatic and terrestrial ecosystem under natural light/dark cycles (a) and exposed to ALAN (b). Artificial illumination can inhibit drift and increases the number of insects emerging from the water under the lamps and increases the flux of aquatic insects into the riparian area through attraction of flying aquatic insects to the light. The community of riparian ground-dwelling predators and scavengers is altered in the lit area likely as a result of the large increase in input of freshwater-derived prey.

Overall, I found evidence that ALAN affects aquatic-terrestrial invertebrate communities through a direct effect on animal dispersal patterns (i.e. macroinvertebrate drift inhibition, aquatic flying insect attraction) as well as an indirect effect (i.e. changes in riparian ground-dwelling secondary consumer taxonomical and dietary composition) caused by increased prey availability (See Fig. 1). The study in Chapter 2 and the two studies in Chapters 3 and 4 were performed in strongly contrasting aquatic environments. The study in Chapter 2 was performed in a fast-flowing sub-alpine stream while the studies in Chapters 3 and 4 in a lowland agricultural ditch with almost no water flow. That similar results were found in both environments indicates that the effect of ALAN is likely widespread to different typology of freshwater ecosystems.

Of the taxa studied, increased density was most pronounced in mayflies of the family Baetidae. This was true for *Baetis* spp. where ALAN inhibited its drifting behaviour in sub-alpine flumes and for *Cloeon* sp. where ALAN increased its emergence in an agricultural ditch. However, the ecological implications of the increased local abundance caused by ALAN for these taxa are still unclear. Baetidae play a major role as grazers, controlling periphyton communities, in many streams (Feminella and Hawkins 1995, Wellnitz et al. 1996). As ALAN increases *Baetis* spp. local densities by drift reduction, one could assume that this might increase grazing pressure on periphyton communities, resulting in reduction of periphyton biomass. However, exposure to ALAN likely results in an inhibition of the overall animal activity, including foraging (Hughes 1966, Bishop 1969). This latency period on a long term, might lead to an increase in the periphyton biomass as well as a decrease of the larval fitness for the Baetidae with an overall decrease in macroinvertebrate local densities. At the same time, drift is regulated by density-dependent interactions (e.g. competition for space) therefore at high densities inhibition of animal drifting behaviour due to ALAN might be overridden (see Chapter 2). As adult, Baetidae are strongly attracted to light sources (Chapter 3). This disrupts their spatial distribution (Perkin et al. 2014a) potentially affecting mayfly swarming and oviposition (Kriska et al. 1998) and eventually increasing mortality by exhaustion (Eisenbeis et al. 2006) (Fig. 2). This might have important implications for the reproductive success and colonization of contiguous freshwater bodies. Larval Baetidae are an important food source for many fish while many terrestrial predators (e.g. arthropods, birds, bats) feed on the adults. Higher aquatic prey availability can lead to changes in the diet of riparian consumers that among others feed on these aquatic species, as I found in Chapters 3 and 4 for ground-dwelling Tetragnathidae (see Fig. 1b). This can be also true for birds, toads, bats and mammals that feed on these subsidies in riparian ecosystems.

ALAN-tolerant invertivorous fish in freshwaters and terrestrial consumers in riparian areas might benefit from the increased number of aquatic prey on the short term. However, the ecological implication of the effect of ALAN on aquatic-terrestrial ecosystems over the long term are still unknown. The ALAN-induced decrease in macroinvertebrate drift and the ALAN-induced increase in aquatic insect emergence and attraction to the light sources (see Fig. 2) of ALAN-sensitive taxa (e.g. Baetidae) might lead to severe decrease in the number of these taxa and to an overall impoverishment of aquatic insect communities in aquatic and riparian ecosystems.

Figure 2. Mayflies cover the ground around a pole of a security light (Millecoquins Point in Naubinway, Michigan). Image courtesy of Phil DeVries.

The effect of ALAN seems to depend on species phenology. In both aquatic and terrestrial communities, we identified periods of high and low ALAN-sensitivity. In sub-alpine streams in periods characterized by low macroinvertebrate density and low basal drift (i.e. spring) ALAN had a strong impact on macroinvertebrate communities. ALAN increased the aquatic insect emergence (Chapter 3) specifically in July and the impact of ALAN on terrestrial arthropods feeding activity varied through the year

(Chapters 3 and 4). I found no evidence that ALAN disrupted the timing of phenological patterns in aquatic insect emergence or in the activity of riparian invertebrates but the strength of the effect varied among seasons. This suggests that the effect of ALAN cannot be assessed without considering other important abiotic factors (e.g. temperature) that regulate animal phenology through the year and might interact with the effect of ALAN.

Freshwater ecosystems and their adjacent areas are subjected to a variety of anthropogenic alterations such as hydrogeomorphological modifications, nutrient enrichment and introduction of invasive species. These contribute to substantial biological degradation of freshwater ecosystems (Schulz et al. 2015, Larsen et al. 2016). ALAN has so far been overlooked. This research has shown that artificial light at night (ALAN) is an important anthropogenic alteration that deserves more attention as it can affect freshwater and adjacent terrestrial ecosystems at different spatial and temporal scales.

5.3 The importance of field experiments

I believe that the question whether ALAN affects natural invertebrate communities cannot be adequately addressed solely using laboratory experiments. Laboratory settings would be too much of an oversimplification to be translatable to the real world. Also, populations analysed in laboratory experiments might not be representative of populations in nature potentially showing unrepresentative ecological effects to a stressor in a natural environment. Therefore, I prioritised patterns of responses observed in large-scale natural scenarios over small-scale experiments conducted in laboratory conditions (Carpenter 1996, Davies and Gray 2015). In this PhD thesis, results were obtained from field experiments in which we introduced artificial illumination to a previously ALAN-naïve area in a controlled manner.

In field experiments, replication is often unfeasible, therefore the trade-off between replication and realism is inevitable. In our study, this was particularly considered in the experiment conducted in the Westhavelland Nature Park in Germany (Chapter 3). This large-scale experimental infrastructure allowed us to study the effect of ALAN in natural environments and at the same time to disentangle the effect of ALAN from other urban confounding effects. Considering the replication-realism trade-off we examined the difference between treatment and control areas before and after the experiment analysing information on pre-disturbance conditions using BACI analyses (Oksanen 2001, Davies and Gray 2015). Furthermore, we monitored a large set of environmental factors across the duration of the experiments including the period prior to the illumination in the control and experimental sites. This was done to ascertain environmental similarity between the two sites and results were discussed and interpreted carefully considering environmental difference when present. All the statistical results were obtained using analytical approaches that accounted for potential spatial and temporal dependency (i.e. random effects). Ultimately, many results obtained in this study are in agreement with previous and ongoing work in other ecosystems and other experiments, strongly suggesting that there are consistent patterns of response in freshwater and terrestrial insect communities.

5.4 Further research

In this work, my observations in the field have raised new research questions that can now be studied in more detail under controlled conditions in order to understand the biological mechanisms behind the observed ecological patterns (see Table 1). At the same time, further research is needed to assess the interaction between ALAN and other stressors. Since the aim of my PhD was to assess the effect of ALAN on ecosystems, the studies of this thesis were conducted using large experimental facilities in which the effect of ALAN was disentangled from other aspects of urbanisation. However, aquatic ecosystems and their adjacent riparian areas are affected by multiple stressors acting in concert (Larsen et al. 2016, Tockner et al*.* 2010) such as increased temperatures, elevated nutrient inputs, hydrogeomorphological alterations and the introduction of invasive species. ALAN might interact with these stressors as multiple stressors often act at the same time. This can be addressed in studies conducted in artificially illuminated mesocosms or already existing anthropogenically-altered areas to integrate a broader picture of the effect of ALAN on urban environments.

Table 1. List of research questions raising from this study

Chapter	ALAN observed effects	New research questions
2	Increased macroinvertebrate abundance and change in community composition	-How does the observed inhibition of drift interact with changes in drift density, i.e. in streams with low levels of drift (e.g., lowland streams)?
	Seasonal effect observed on macroinvertebrates	- Are larval developmental stages differently affected by ALAN? - Is competition for resources overruling the effect of ALAN?
3	Increased aquatic insect emergence	- Is ALAN decreasing macroinvertebrate predatory pressure from invertivorous fish? - Are aquatic insect larvae attracted to light sources? - Is the increased insect emergence the results of decreased larval drift?
	Attraction of aquatic insects to light sources	- Is ALAN shortening or increasing the distance that organisms can move away from the water, thus modifying natural stream width and the river´s biological signature in the wider landscape (sensu Muehlbauer et al. 2014, Gurnell et al. 2016)?

This study also raises the need for studies analysing the effect of LEDs on ecosystems (see Paragraph 5.3). In fact, LEDs, and particularly white LEDs with a large peak in the blue, may have even greater effects on nocturnal invertebrates than high-pressure sodium (HPS) lights given the sensitivity of many organisms to short wavelength light (van Langevelde et al. 2011, van Grunsven et al. 2014, Pawson and Bader 2014). Due to the ongoing large-scale replacement of HPS with LED technology in many cities, an interdisciplinary effort is essential to advance scientific understanding of the ecological consequences of those new lighting types on wild organisms.

Further studies assessing the effect of ALAN should be performed in different types of aquatic ecosystems since the effect of ALAN may vary in different freshwater habitats. For instance, in some habitats such as low-land streams (e.g. agricultural ditches) drift is nearly absent while in running streams this plays an important role in dispersal of macrofauna. Therefore, in lowland streams the attraction of adult and larval aquatic insects to ALAN might play a major role while in running stream inhibition of drift might be more important. Furthermore, in lowland streams, aquatic invertebrates can aggregate under illuminated areas and, in absence of density-dependent dispersal drift, this might result in higher emergence rate compared to running streams, with stronger effects on cross-ecosystem aquatic subsidy dynamics.

Further investigation of the effect of ALAN should be conducted on Baetidae mayflies. I found Baetidae to be particularly sensitive to ALAN both as larvae (*Baetis* spp.) and adults (*Cloeon* sp.) (see Paragraph 5.2). However, other more rare species may have been missed and the biological mechanisms behind the ecological patterns observed in this taxon need to be better understood (see Paragraph 5.2). Because they are globally widespread in freshwaters and known to be sensitive to environmental change and degradation, Baetidae are used as bio-indicator. My results suggest they would also be suitable indicators of ALAN stress in restoration and biomonitoring programs on aquatic ecosystems and their adjacent riparian areas.

5.5 Implications for policy and management

Given the abundance of streetlights along streams and rivers and along the shores of lakes, reservoirs, and wetlands, the ecological changes observed in this research at the local scale, are likely to extend to larger spatial scales. Due to the important role of aquatic subsidies to consumers in recipient ecosystems, the impact of artificial illumination has to be considered as a relevant stressor, in urban and landscape planning. I observed that exposure to ALAN caused a disproportionate availability for aquatic prey (Chapter 3) and that this resulted in consumer dietary shifts (Chapter 4). This might release predatory pressure from other prey species causing a change of predator-prey dynamics. In the case of semi-urban and agricultural areas, this might have severe consequence for the natural control by predation of invertebrate pest populations (e.g. Aphidae, Auchenorrhyncha) (Dixon 2000, Hassell 1978, Polis and Strong 1996) which can form a serious threat for agricultural production, if not controlled (Dedryver et al. 2010).

I demonstrated that both light-emitting diode (LED) and high-pressure sodium (HPS) lamps had an effect on aquatic and terrestrial ecosystems, although they are both considered “environmental friendly” (Li 2010) and “insect friendly” (Eisenbeis et al. 2006). Because LED is one of the most energy efficient lighting techniques, many countries are switching to LEDs (Perkin et al. 2011) Although its effects of LEDs on ecosystems are still largely unknown there are indications that they can have a negative impact (see van Langevelde et al. 2011, van Grunsven et al. 2014, Pawson and Bader 2014, Chapter 2). Therefore, the implications that this light technology might have for ecology, should be carefully considered in lighting policy and landscape planning (s.a. Schroer and Hölker 2016).

Ecological impacts should be considered in the design of new lighting concepts especially when illumination is placed near freshwater ecosystems. This requires substantive interdisciplinary efforts by landscape and urban planners, lighting engineers, and terrestrial and aquatic ecologists to advance scientific understanding and to use these advances to improve restoration and management of aquatic-terrestrial habitats. The installation of artificial lights directly adjacent to stream riverbanks should be avoided or, if necessary, carefully designed to limit the impact as much as possible. This can be done, for example, by establishing adequate spatial and temporal riparian buffers to protect movement and dispersal of both aquatic and terrestrial organisms. ALAN should only be directed to where it is needed, in the lowest intensity required for its use and only when necessary.

5.6 References

Baxter, C. V., K. D. Fausch, and W. Carl Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology, **50**: 201-220.

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

Carpenter, S. R. 1996. Microcosm experiments have limited relevance for community and ecosystem ecology. Ecology **77**: 677-680.

Carpenter, S. R., E. H. Stanley, and M. J. Vander Zanden. 2011. State of the world's freshwater ecosystems: physical, chemical, and biological changes. Annual Review of Environment and Resources **36**: 75-99.

Davies, G. M., and A. Gray, 2015. Don't let spurious accusations of pseudoreplication limit our ability to learn from natural experiments (and other messy kinds of ecological monitoring). Ecology and Evolution **5**: 5295-5304.

Dedryver, C. A., A. Le Ralec, and F. Fabre. 2010. The conflicting relationships between aphids and men: a review of aphid damage and control strategies. Comptes Rendus Biologies **333**: 539-553.

Dijkstra, K. D. B., M. T. Monaghan, and S. U. Pauls. 2014. Freshwater Biodiversity and Insect Diversification. Annual Review of Entomology **59**: 143-163

Dixon, A. F. G. 2000. Insect predator-prey dynamics: ladybird beetles and biological control. - Cambridge University Press.

Eisenbeis, G., C. Rich, and T. Longcore. 2006. Artificial night lighting and insects: attraction of insects to streetlamps in a rural setting in Germany. Pages 191-198 *in* Rich G. C. and Longcore T. editors. Ecological consequences of artificial night lighting. Island Press, Washington DC, USA.

Feminella, J. W., and C. P. Hawkins. 1995. Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. Journal of the North American Benthological Society **14**: 465-509.

Gratton, C., and M. J. Vander Zanden. 2009. Flux of aquatic insect productivity to land: comparison of lentic and lotic ecosystems. Ecology **90**: 2689-2699.

Gurnell, A. M., W. Bertoldi, K. Tockner, G. Wharton, and G. Zolezzi. 2016. How large is a river? Conceptualizing river landscape signatures and envelopes in four dimensions. Wiley Interdisciplinary Reviews: Water **3**: 313-325.

Hassell, M. P. 1978. The dynamics of arthropod predator-prey systems. - Princeton University Press.

Hughes, D. 1966. On the dorsal light response in a mayfly nymph. Animal Behaviour **14:** 13-16.

Jackson, R. B., S. R. Carpenter, C. N. Dahm, D. M. McKnight, R. J. Naiman, S. L. Postel, and S. W. Running. 2001. Water in a changing world. Ecological Applications **11**: 1027-1045.

Kriska, G., G. Horváth, and S. Andrikovics. 1998. Why do mayflies lay their eggs en masse on dry asphalt roads? Water-imitating polarized light reflected from asphalt attracts Ephemeroptera. Journal of Experimental Biology **201**: 2273-2286.

Larsen, S., J. D. Muehlbauer, and E. Marti. 2015. Resource subsidies between stream and terrestrial ecosystems under global change. Global Change Biology **22**: 2489–2504.

Li, X. 2010. U.S. Patent No. 7,832,898. Washington, DC: U.S. Patent and Trademark Office.

Muehlbauer, J. D., S. F. Collins, M. W. Doyle, and K. Tockner. 2014. How wide is a stream? Spatial extent of the potential “stream signature” in terrestrial food webs using meta-analysis. Ecology **95**: 44-55.

Oksanen, L. 2001. Logic of experiments in ecology: is pseudoreplication a pseudoissue? Oikos **94**: 27-38.

Pawson, S., and M.-F. Bader. 2014. LED lighting increases the ecological impact of light pollution irrespective of color temperature. Ecological Applications **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. Freshwater Biology **59**: 368-377.

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

Pimm, S. L. 1984. The complexity and stability of ecosystems. Nature **307**: 321-326.

Polis, G. A. and Strong, D. R. 1996. Food web complexity and community dynamics. American Naturalist **147**: 813-846.

Power, M. E., W. E. Rainey, M. S. Parker, J. L. Sabo, A. Smyth, S. Khandwala, J. C. Finlay, F. C. McNeely, K. Marsee, and C. Anderson. 2004. River-to-watershed subsidies in an old-growth conifer forest. Pages 217-240 *in* Polis G. A., Power M. E. and Huxel G. R editors. Food webs at the landscape level. The University of Chicago Press.

Richardson, J. S., Y. Zhang, and L. B. Marczak. 2010. Resource subsidies across the land–freshwater interface and responses in recipient communities. River Research and Applications **26**: 55-66.

Schroer, S., and F. Hölker. 2016. Impact of lighting on flora and fauna. Pages 1-33 *in* Karlicek et al. editors. Handbook of Advanced Lighting Technology Reference. Springer International Publishing.

Schulz, R., M. Bundschuh, R. Gergs, C. A. Brühl, D. Diehl, M. H. Entling, L. Fahse, O. Frör, H. F. Jungkunst, A. Lorke, and R. B. Schäfer. 2015. Review on environmental alterations propagating from aquatic to terrestrial ecosystems. Science of the Total Environment **538**: 246-261.

Tockner, K., M. Pusch, D. Borchardt and M. S. Lorang. 2010. Multiple stressors in coupled river–floodplain ecosystems. Freshwater Biology, **55**: 135-151.

van Grunsven, R. H. A., M. Donners, K. Boekee, I. Tichelaar, K. Van Geffen, D. Groenendijk, F. Berendse, and E. Veenendaal. 2014. Spectral composition of light sources and insect phototaxis, with an evaluation of existing spectral response models. Journal of Insect Conservation **18**: 225-231.

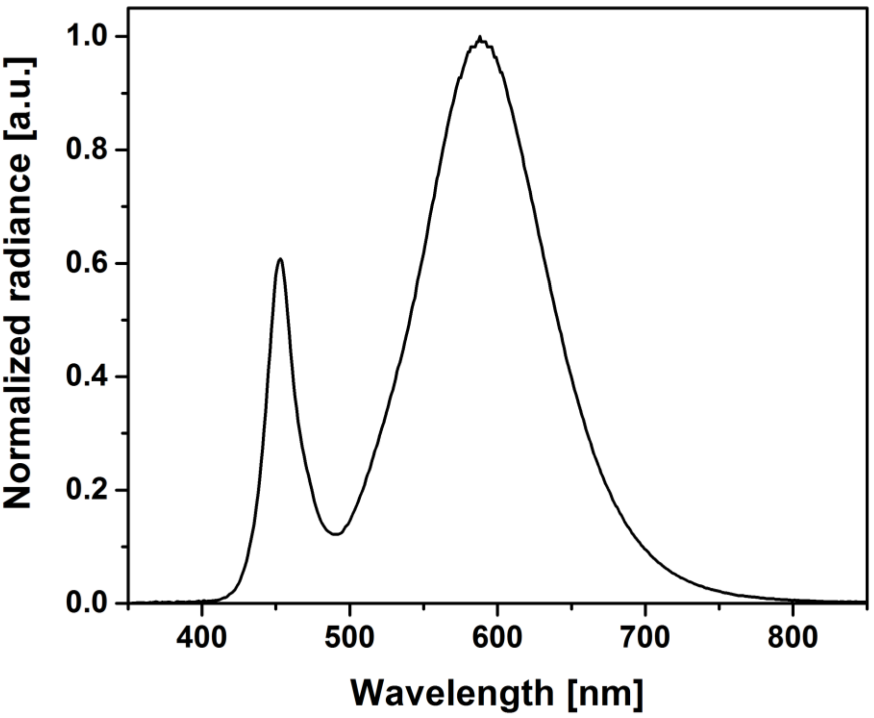
van Langevelde, F., J. A. Ettema, M. Donners, M. F. WallisDeVries, and D. Groenendijk. 2011. Effect of spectral composition of artificial light on the attraction of moths. Biological Conservation **144**: 2274-2281.

Wellnitz, T. A., R. B. Rader, and J. V. Ward. 1996. Light and a grazing mayfly shape periphyton in a Rocky Mountain stream. Journal of the North American Benthological Society **15**: 496-507.

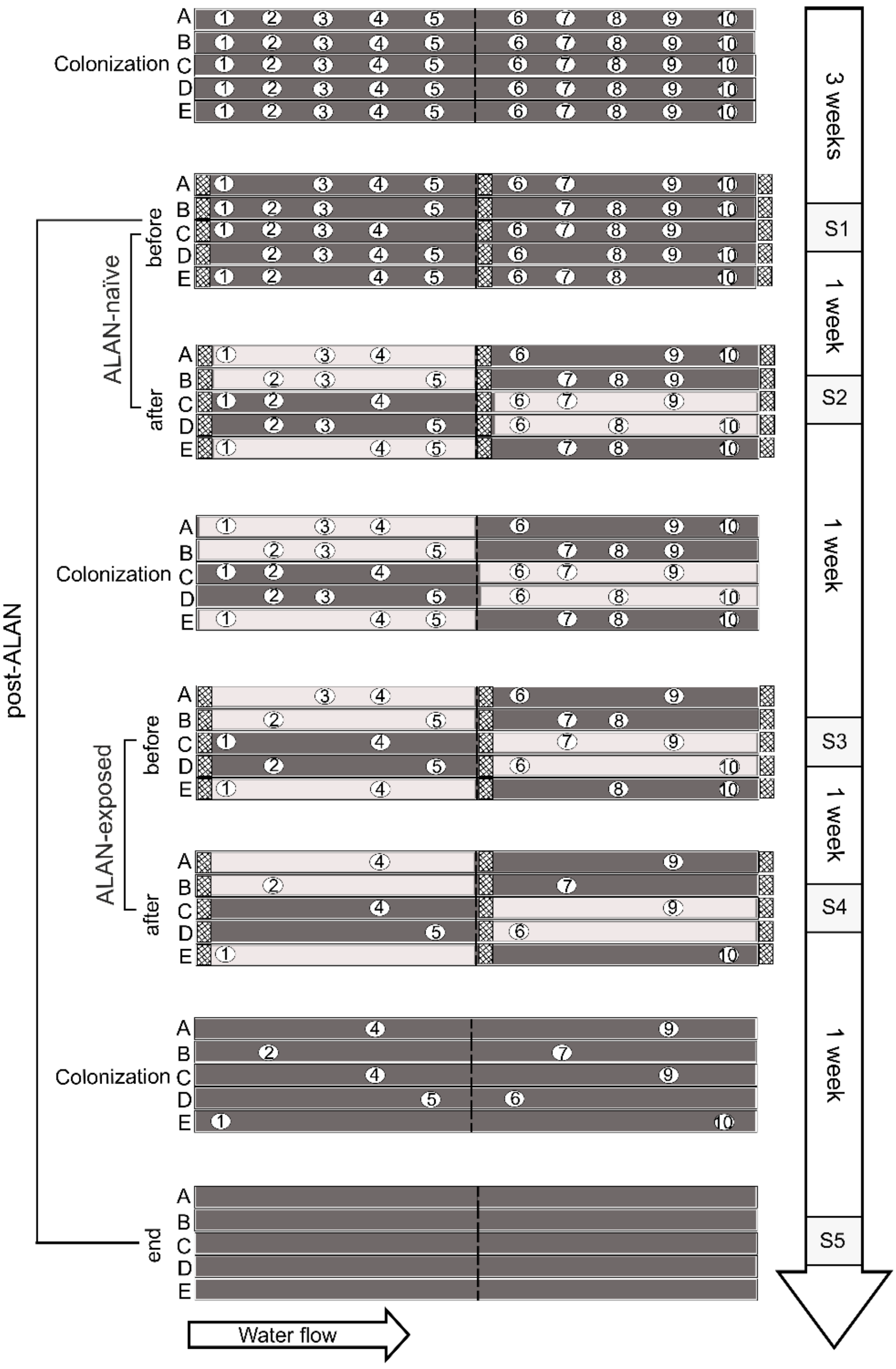
Appendix

Chapter 2

Appendix S1. Spectral composition of LED lights used in the study (12 V, 3004 K, Barthelme, Nürnberg, Germany).



Appendix S2. Scheme of the complete experimental design representing the five flumes (A, B, C, D, E) and the baskets used to sample macroinvertebrates. The illustration is chronologically organized from the top to the bottom. The arrow on the right side shows the time elapsed between sampling events (S1, S2, S3, S4, S5); the corresponding statistical analyses are indicated on the left side: for ALAN-naïve, between "before" and "after" periods; for ALAN-exposed, between "before" and "after" periods; for communities after the end of the illumination (post-ALAN) between "end" and "ALAN-naïve, before" periods. Drift nets are indicated in the illustration when and where used to isolate the flumes.



Appendix S3. List of taxa collected during the experiment in spring and autumn 2013. For each taxa is shown the total density (individuals per m^2) in treatment and control sections.

Taxa	Stadium	Density (Spring)		Density (Autumn)	
		Treatment	Control	Treatment	Control
Amphinemura sp.	Larvae	6.59	2.41	0.00	0.00
Arcynopterix sp.	Larvae	0.59	0.00	0.00	0.00
Baetis spp.	Larvae	973.76	498.88	15501.65	14857.67
Blephariceridae	Larvae	0.00	0.00	3.66	2.48
Brachiptera risi	Larvae	182.93	199.13	0.00	0.00
Capnia sp.	Larvae	9.06	12.45	0.00	0.00
Chloroperla tripunctata	Larvae	1.38	0.00	0.00	0.00
Dinoscras sp.	Larvae	0.00	0.87	0.00	0.00
Ecdyonurus sp.	Larvae	6.78	3.34	8.34	14.59
Epeorus sp.	Larvae	13.35	2.06	205.17	189.50
Heptagenia sp.	Larvae	2.24	0.00	18.68	0.00
Heptageniidae	Larvae	0.00	0.74	0.00	0.00
Hydrophilus sp	Adult	0.00	0.00	0.00	0.88
Hydropsychidae	Larvae	833.41	619.75	1910.50	1085.90
Isoperla sp.	Larvae	4.30	1.96	2.17	2.36
Leptophlebia sp.	Larvae	0.72	0.00	4.81	0.89
Leptophlebidae	Larvae	0.00	0.00	1.45	0.88
Leuctra sp.	Larvae	62.16	27.25	399.69	271.97
Limnephilus sp.	Larvae	0.00	0.61	1.77	4.33
Nemoura sp.	Larvae	13.76	22.38	205.24	153.72
Odontoceridae	Larvae	0.00	0.00	7.17	0.88
Perla sp.	Larvae	0.00	0.71	0.00	0.00
Perlodes sp.	Larvae	1.50	2.16	3.01	3.49
Phylopotamidae	Larvae	1.52	0.00	0.00	0.00
Procleon sp.	Larvae	2.80	0.00	0.00	0.00
Protonemura sp.	Larvae	92.31	60.42	5.72	14.10
Rhithrogena sp.	Larvae	10.03	20.52	4.68	9.96
Rhyacophila sp.	Larvae	11.78	3.38	286.28	185.84
Sericostoma sp.	Larvae	1.47	0.89	6.68	16.05
Serratella sp.	Larvae	0.00	0.00	3.79	2.53
Siphonoperla sp.	Larvae	14.10	7.91	4.83	0.89
Trichoptera sp.	Larvae	0.00	0.00	1.57	0.00
Ancylus sp.	Larvae	0.00	0.00	5.57	3.71
Asellus sp.	Adult	0.83	0.00	0.69	0.00

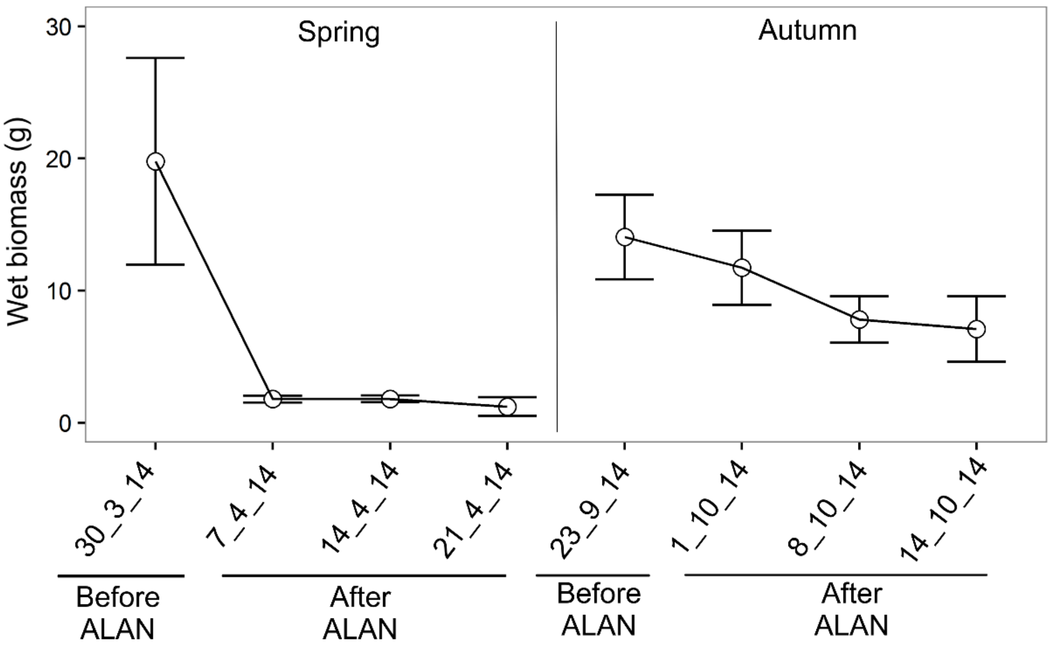
Athericidae	Larvae	5.28	5.95	3.60	3.17
Atherix ibis	Larvae	1.36	0.00	0.00	0.00
Ceratopogonidae	Larvae	0.93	0.00	0.00	0.00
Chironomidae	Larvae	4883.68	2735.42	1948.75	1087.11
Coleoptera	Larvae	1.61	0.62	0.00	0.00
Crenobia alpina	Larvae	0.00	0.00	0.79	0.83
Diptera	Larvae	3.34	1.63	0.86	0.00
Dixidae	Larvae	0.00	0.73	0.98	0.00
Elmidae	Larvae	31.03	26.66	33.09	26.48
Elmidae	Adult	5.42	3.73	7.25	5.16
Empididae	Larvae	3.98	0.00	0.92	0.00
Ephydridae	Larvae	0.00	0.62	0.00	2.34
Gordioidei	Adult	0.00	0.00	0.61	0.00
Hydracarina	Adult	19.41	19.52	183.86	158.17
Hydraenidae	Larvae	0.00	0.00	0.00	2.47
Hydraenidae	Adult	1.52	0.00	4.44	0.83
Limoniidae	Larvae	0.72	0.00	1.35	0.80
Lymnaea sp.	Adult	0.00	0.61	0.00	0.89
Nematomorpha	Adult	0.67	0.00	0.77	0.00
Planaria alpina	Adult	0.00	0.00	8.34	2.31
Psychodidae	Larvae	0.83	0.00	0.00	0.00
Simuliidae	Larvae	1933.66	1356.92	11539.14	10267.96
Turbellaria	Adult	0.00	0.00	0.88	1.57

Appendix S4. Environmental parameters measured in the flumes for the two seasons (n = 20).

Spring	31.03. (before ALAN)					07.04. (after one week)				
Flume	A	B	C	D	E	A	B	C	D	E
Conductivity (μS cm^{-2})	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^{-2})	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^{-2})	NA	NA	NA	NA	NA	0.3	0.2	0.3	0.2	0.3
Date	14.04. (two weeks)					23.04. (three weeks)				
Flume	A	B	C	D	E	A	B	C	D	E
Conductivity (μS cm^{-2})	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^{-2})	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^{-2})	0.5	0.4	0.3	0.3	0.4	0.5	0.4	0.3	0.3	0.3

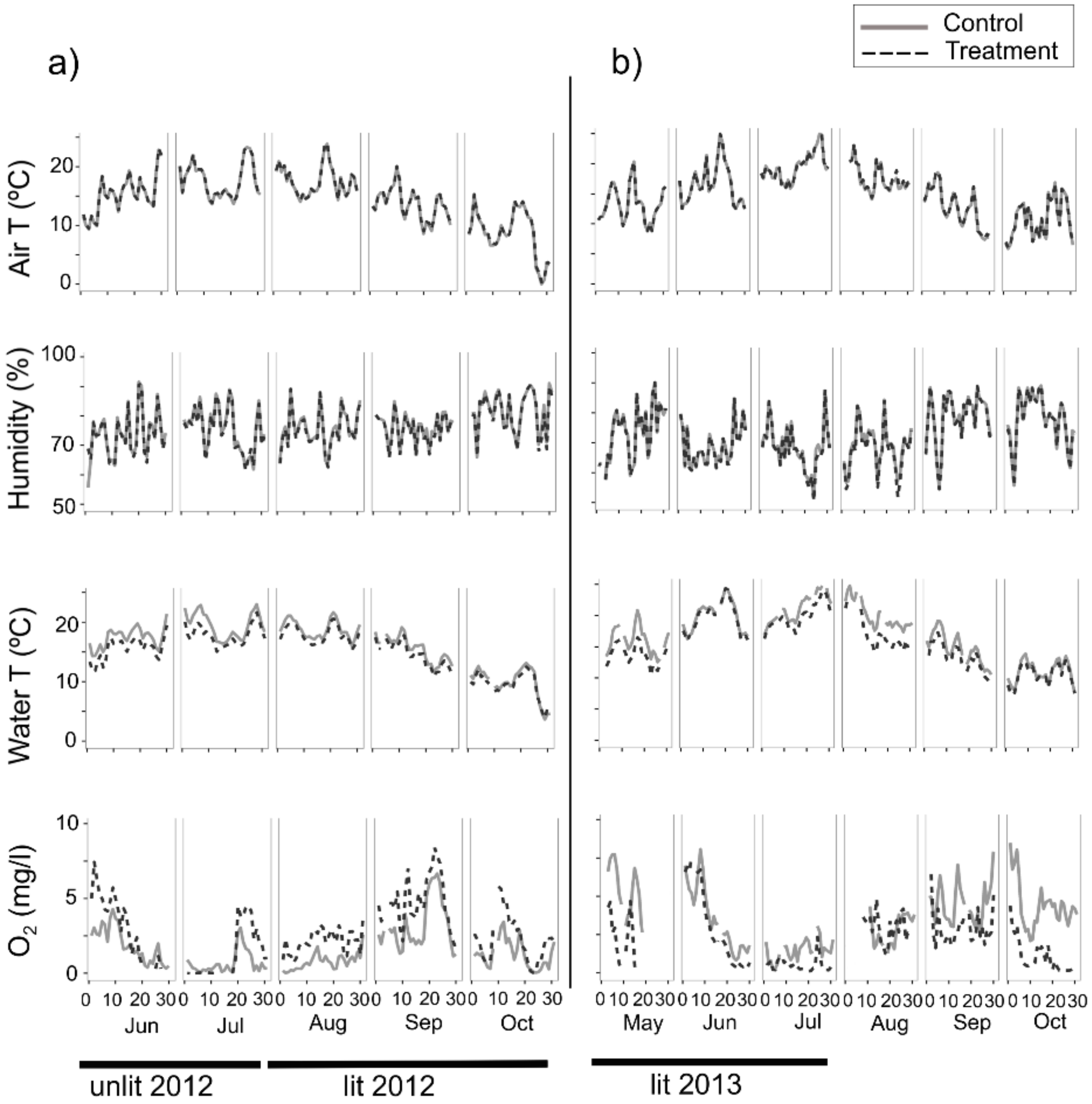
Autumn	24.09. (before)					01.10. (after one week)				
Flume	A	B	C	D	E	A	B	C	D	E
Conductivity (μS cm^{-2})	143.6	143.2	143.1	143.1	143.1	147.6	147.7	147.7	147.6	147.6
Temperature (°C)	10.7	10.65	10.7	10.7	10.05	12.8	12.8	12.8	12.8	12.8
Oxygen (mg L^{-2})	9.18	9.23	9.36	9.31	9.72	10.10	10.11	10.55	10.68	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^{-2})	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. (three weeks)				
Flume	A	B	C	D	E	A	B	C	D	E
Conductivity (μS cm^{-2})	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^{-2})	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^{-2})	0.4	0.4	0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.4

Appendix S5. Wet biomass of incoming drift measured in the drift nets placed at the upstream sluice gate. Mean value and ± standard deviation (as errorbars) are shown for sampling dates before and after exposure to artificial light at night (ALAN) in spring and autumn 2014.



Chapter 3

Appendix S6. Environmental factors at the treatment and control site in 2012 (a) and 2013 (b) among months and BACI periods (“unlit 2012”; “lit 2012”; “lit 2013”). In the plots mean daily values for air temperature, relative humidity, water temperature and oxygen concentration in the water are shown.



Appendix S7. Comparison for selected environmental variables using Generalized Least Squares analysis to test difference between treatment and control site within each period of the BACI analysis (unlit 2012, lit 2012, lit 2013) and for each month (May until October 2013) for the analysis of the temporal patterns. *F*-statistic and significance (p) are shown.

Variable	Periods/Months	F-statistic	*p*	Mean Control	Mean Treatment
Air temperature (°C)	May-Jul 2012 (unlit 2012)	$F_{1,120}$=0.26	0.6	16.5	16.6
	Jul-Oct 2012 (lit 2012)	$F_{1,182}$=3.60	0.06	13.3	13.4
	May-Jul 2013 (lit 2013)	$F_{1,182}$=0.24	0.62	16.5	16.5
	May 2013	$F_{1,60}$=0.39	0.53	13.3	13.3
	Jun 2013	$F_{1,58}$=0.09	0.75	16.7	16.7
	Jul 2013	$F_{1,60}$=0.007	0.93	19.5	19.5
	Aug 2013	$F_{1,60}$=2.43	0.12	18.1	18.3
	Sep 2013	$F_{1,58}$=1.58	0.21	12.7	12.8
	Oct 2013	$F_{1,60}$=0.03	0.84	10.8	10.9
Humidity (%)	May-Jul 2012 (unlit 2012)	$F_{1,120}$=0.80	0.37	74.8	74.4
	Jul-Oct 2012 (lit 2012)	$F_{1,182}$=3.57	0.06	77.9	77.3
	May-Jul 2013 (lit 2013)	$F_{1,182}$=0.56	0.45	70.1	69.8
	May 2013	$F_{1,60}$=0.12	0.72	72.9	72.9
	Jun 2013	$F_{1,58}$=0.10	0.74	68.9	68.6
	Jul 2013	$F_{1,60}$=0.59	0.44	68.6	68.0
	Aug 2013	$F_{1,60}$=3.82	0.06	68.9	67.8
	Sep 2013	$F_{1,58}$=0.36	0.54	78.8	78.4
	Oct 2013	$F_{1,60}$=0.06	0.79	77.9	77.7
Water T (°C)	May-Jul 2012 (unlit 2012)	$F_{1,120}$=200.58	<0.001	18.5	16.7
	Jul-Oct 2012 (lit 2012)	$F_{1,182}$=78.66	<0.001	14.7	13.9
	May-Jul 2013 (lit 2013)	$F_{1,182}$=56.11	<0.001	19.1	17.7
	May 2013	$F_{1,60}$=149.70	<0.001	16.0	13.7
	Jun 2013	$F_{1,58}$=27.93	<0.001	20.0	19.6
	Jul 2013	$F_{1,60}$=35.77	<0.001	21.2	19.9
	Aug 2013	$F_{1,60}$=91.20	<0.001	20.1	17.5
	Sep 2013	$F_{1,58}$=134.68	<0.001	14.7	13.3
	Oct 2013	$F_{1,60}$=29.78	<0.001	11.0	10.5
Oxygen (mg l^{-1})	May-Jul 2012 (unlit 2012)	$F_{1,120}$=3.99	0.05	1.3	2.0

Jul-Oct 2012 (lit 2012)	$F_{1,182}=83.85$	<0.001	1.8	3.1
May-Jul 2013 (lit 2013)	$F_{1,182}=9.34$	0.003	3.4	1.9
May 2013	$F_{1,60}=17.98$	<0.001	5.1	2.0
Jun 2013	$F_{1,58}=1.14$	0.29	3.5	3.1
Jul 2013	$F_{1,60}=25.26$	<0.001	1.5	0.5
Aug 2013	$F_{1,60}=0.51$	0.47	2.7	2.5
Sep 2013	$F_{1,58}=22.65$	<0.001	4.5	3.1
Oct 2013	$F_{1,60}=694.65$	<0.001	4.4	1.0

Appendix S8. Taxa list for emergence, air eclector and pitfall traps for the entire study.

Trap	Order	Family	*Genus*	*Species*
Emergence	Diptera	Dixidae		
		Ephydridae		
		Cecidomyiidae		
		Chaoboridae		
		Chironomidae		
		Culicidae		
		Dixidae		
		Dolichopodidae		
		Drosophilidae		
		Empididae		
		Ephydridae		
		Lonchopteridae		
		Muscidae		
		Phoridae		
		Psychodidae		
		Sciaridae		
		Simuliidae		
	Ephemeroptera	Baetidae	*Cloeon*	*dipterum*
	Odonata	Coenagrionidae	*Ischnura*	*elegans*
		Lestidae	*Lestes*	*sponsa*
	Trichoptera	Hydroptilidae		
		Leptoceridae		
		Polycentropodidae		
		Brachycentridae		
		Leptoceridae		
Air Eclector	Acari			
	Aranea			
	Auchenorrhyncha			
	Blattoptera			
	Brachycera			
	Coleoptera	Anthicidae	*Notoxus*	*monoceros*
		Apionidae		
		Carabidae		
		Dytiscidae		
		Staphylinidae		
		Cerambycidae		
		Chrysomelidae		

	Coccinellidae	*Calvia*	*quatuorderc.*
		Harmonia	*axyridis*
	Hydrophilidae	*Hydrobius*	*fuscipes*
	Scarabaeidae	*Aphodius*	sp.
	Curculionidae		
	Staphylinidae		
	Tenebrionidae	*Tenebrio*	*molitor*
	Trogidae	*Trox*	sp.
Ephemeroptera	Baetidae	*Cloeon*	*dipterum*
Lepidoptera	Arctiidae	*Cascinia*	*cribraria*
		Eilema	*lurideola*
		Arctia	*caja*
		Eilema	*lurideola*
		Miltochrista	*miniata*
		Phragmatobia	*fuliginosa*
		Rhyparia	*purpurata*
		Spilosoma	*lubricipeda*
			lutea
			urticae
	Cossidae	*Phragmataecia*	*castaneae*
	Drepanidae	*Drepana*	*falcataria*
	Endromidae	*Endromis*	*versicolora*
	Geometridae	*Cidaria*	*galiata*
		Pseudeustrotia	*candidula*
		Angerona	*prunaria*
		Biston	*strataria*
		Cabera	*pusaria*
		Campaea	*margaritata*
		Charissa	*ambiguata*
		Chiasmia	*clathrata*
		Ennomos	*autumnaria*
			erosaria
		Epirrhoe	*alternata*
		Erannis	*defolaria*
		Geometra	*papilionaria*
		Hemithea	*aestivaria*
		Hypomecis	*punctinalis*
			roboraria
		Idaea	*deversaria*
		Lithostege	*grisaeata*

	Lomaspilis	*marginata*
	Lycia	*hirtaria*
	Scopula	*caricaria*
		immorata
	Selenia	*tetralunaria*
	Timandra	*comae*
Crambidae		
Lasiocampidae	*Dendrolimus*	*pini*
	Lasiocampa	*trifolii*
	Macrothylacia	*rubi*
	Euthrix	*potatoria*
	Gastropacha	*quercifolia*
	Malacosoma	*neustria*
	Poecilocampa	*populi*
Lymantriidae	*Gynaephora*	*fascelina*
	Sphrageidus	*similis*
Melyridae	*Dasytes*	sp.
Noctuidae	*Diarsia*	*mendica*
	Diarsia	*rubi*
	Eugnorisma	*glareosa*
	Globia	*algae*
	Globia	*algae*
	Hadena	*confusa*
	Hoplodrina	*blanda*
	Mniotype	*satura*
	Mythimna	*albipuncta*
	Naenia	*typica*
	Panthea	*coenobita*
	Phlogophora	*meticulosa*
	Staurophora	*celsia*
	Xestia	*ditrapezium*
	Xestia	*triangulum*
	Acontia	*trabealis*
	Acronicta	*rumicis*
	Agrochola	*litura*
	Agrotis	*exclamationis*
	Allophyes	*oxyacanthae*
	Amphipoea	*fucosa KOM.*
	Amphipyra	*tragoponis*
	Apamea	*lateritia*

	monoglypha
	remissa
	unanimis
Asteroscopus	*sphinx*
Autographa	*gamma*
Axylia	*putris*
Ceramica	*pisi*
Cerapteryx	*graminis*
Cerastis	*rubricosa*
Deltote	*bankiana*
Diachrysia	*chrysitis*
Diarsia	*rubi*
Eucarta	*virgo*
Eupsilia	*transversa*
Hadena	*bicruris*
Helotropha	*leucostigma*
Hoplodrina	*octogenaria*
Hydraecia	*micacea*
Hypena	*proboscidalis*
Ipimorpha	*subtusa*
Lacanobia	*suasa*
	W-latinum
Lateroligia	*ophiogramma*
Mesapamea	*secalis*
Mythimna	*ferrago*
	impura
	turca
Noctua	*pronuba*
Ochopleura	*plecta*
Oria	*musculosa*
Orthosia	*cruda*
	gothica
	gracilis
	opima
Paracolax	*tristalis*
Photedes	*extrema*
Phragmatiphila	*nexa*
Plusia	*festucae*
Pseudeustrotia	*candidula*
Rhizedra	*lutosa*

	Rivula	*sericealis*
	Simyra	*albovenosa*
	Tholera	*decimalis*
	Xestia	*C-nigrum*
		sexstrigata
	Xylena	*vetusta*
Notodontidae	*Furcula*	*bicuspis*
	Drymonia	*querna*
	Notodonta	*dromedarius*
	Phalera	*bucephala*
	Ptilodon	*capucina*
Nymphalidae	*Inachis*	*io*
Pyralidae	*Dioryctria*	*abietella*
	Nomophila	*noctuella*
	Apomyelois	*bistiatella*
	Cataclysta	*lemnata*
	Catopria	sp.
	Chilo	*phragmitella*
	Chrysoteuchia	*culmella*
	Elophila	*nympheata*
	Evergestis	*extimalis*
	Loxostege	*sticticalis*
	Nymphula	*nitidulata*
	Ostrinia	*nubilalis*
	Paraponyx	*stratiotata*
	Platytes	*alpinella*
	Pleuroptya	*ruralis*
	Scoparia	sp.
	Synaphe	*punctalis*
Pyraloidea		
Sphingidae	*Deilephila*	*elpenor*
	Laothoe	*populi*
	Sphinx	*pinastri*
Thaumetopoeidae	*Thaumetopoea*	*processionea*
Tortricidae	*Acleris*	sp.
	Aethes	*cnicana*
		smeathmanniana
	Agapeta	*hamana*
	Bactra	sp.
	Celypha	*lacunana*

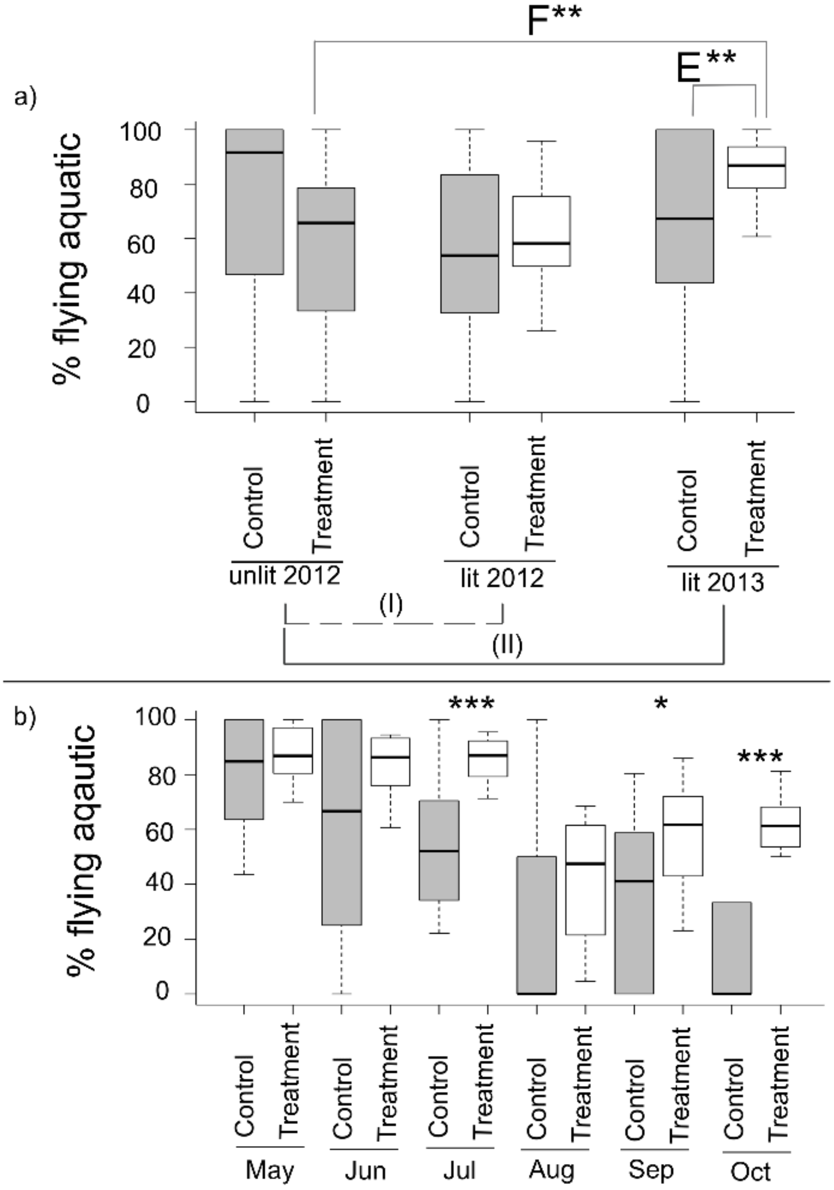
				woodiana
			Cnephasia	*incertana*
				longana
			Eana	*argentana*
			Lathronympha	*strigana*
			Loxoterma	*rivulana*
			Exapate	*congelatella*
	Microlepidoptera			
	Mecoptera			
	Megaloptera			
	Nematocera			
	Neuroptera			
	Psocoptera			
	Diptera			
	Hemiptera			
	Psocoptera			
	Trichoptera			
Pitfall	Annelida			
	Aranea	Clubionidae		
		Corinnidae	*Phrurolithus*	*festivus*
		Gnaphosidae	*Gnaphosa*	*bicolor*
		Gnaphosidae	*Micaria*	*pulicaria*
		Gnaphosidae	*Zelotes*	*electus*
		Liocranidae	*Liocranoeca*	*striata*
		Pisauridae	*Dolomedes*	*fimbriatus*
		Salticidae		
		Theridiidae	*Euryopis*	*flavomaculata*
		Thomisidae	*Xysticus*	*cristatus*
		Thomisidae	*Xysticus*	*kochi*
		Zoridae	*Zora*	*spinimana*
		Theridiidae		
		Linyphiidae	*Abacoproeces*	*saltuum*
			Allomengea	*vidua*
			Bathyphantes	*approximatus*
				gracilis
			Centromerita	*bicolor*
			Dendryphantes	sp.
			Dicymbium	*nigrum brevisetosum*
			Diplostyla	*concolor*
			Erigone	*atra*

			dentipalpis
		Gnathonarium	*dentatum*
		Gongylidiellum	*mucidans*
		Meioneta	*cf affinis*
		Oedothorax	*apicatus*
			fuscus
			retusus
		Savignia	*frontata*
		Tenuiphantes	*tenuis*
	Lycosidae	*Alopecosa*	sp.
		Arctosa	*leopardus*
		Pardosa	*amentata*
		Pardosa	*lugubris*
		Pardosa	*monticola*
		Pardosa	*paludicola*
		Pardosa	*prativaga*
		Pardosa	*pullata*
		Pardosa	sp.
		Pirata	*piraticus*
		Pirata	*uliginosus*
		Piratula	*hygrophila*
		Piratula	*latitans*
		Throcosa	sp.
		Trochosa	*ruricola*
	Tetragnathidae	*Pachygnatha*	*clercki*
		Tetragnatha	sp.
			degeeri
Auchenorrhyncha	Cicadellidae	*Cicadella*	*cicadella*
		Cicadella	*viridis*
	Delphacidae		
Caelifera			
Coleoptera	Carabidae	*Agonum*	*duftschmidi*
		Anisodactylus	*binotatus*
		Anthracus	*consputus*
		Badister	*collaris*
		Badister	*dilatatus*
		Badister	*meridionalis*
		Badister	*sodalis*
		Badister	*unipustulatus*
		Bembidion	*assimile*

	Bembidion	*biguttatum*
	Bembidion	*gilvipes*
	Bembidion	*guttula*
	Bembidion	*properans*
	Blethisa	*multipunctata*
	Calathus	*melanocephalus*
	Carabus	*convexus*
	Carabus	*granulatus*
	Carabus	*nemoralis*
	Chlaenius	*nigricornis*
	Cychrus	*caraboides*
	Dyschirius	*globosus*
	Elaphrus	*cupreus*
	Harpalus	*rufipes*
	Leistus	*ferrugineus*
	Leistus	*terminatus*
	Loricera	*pilicornis*
	Nebria	*brevicollis*
	Notiophilus	*palustris*
	Oodes	*helopioides*
	Oxypsephalus	*obscurus*
	Panagaeus	*crux-major*
	Patrobus	*atrorufus*
	Platynus	*livens*
	Poecilus	*cupreus*
	Poecilus	*versicolor*
	Pterostichus	*diligens*
	Pterostichus	*gracilis*
	Pterostichus	*melanarius*
	Pterostichus	*minor*
	Pterostichus	*niger*
	Pterostichus	*nigrita*
	Pterostichus	*strenuus*
	Pterostichus	*vernalis*
	Stenolophus	*mixtus*
	Syntomus	*truncatellus*
	Synuchus	*vivalis*
	Trechus	*quadristriatus*
Cholevidae		
Chrysomelidae	*Chaetocnema*	sp.

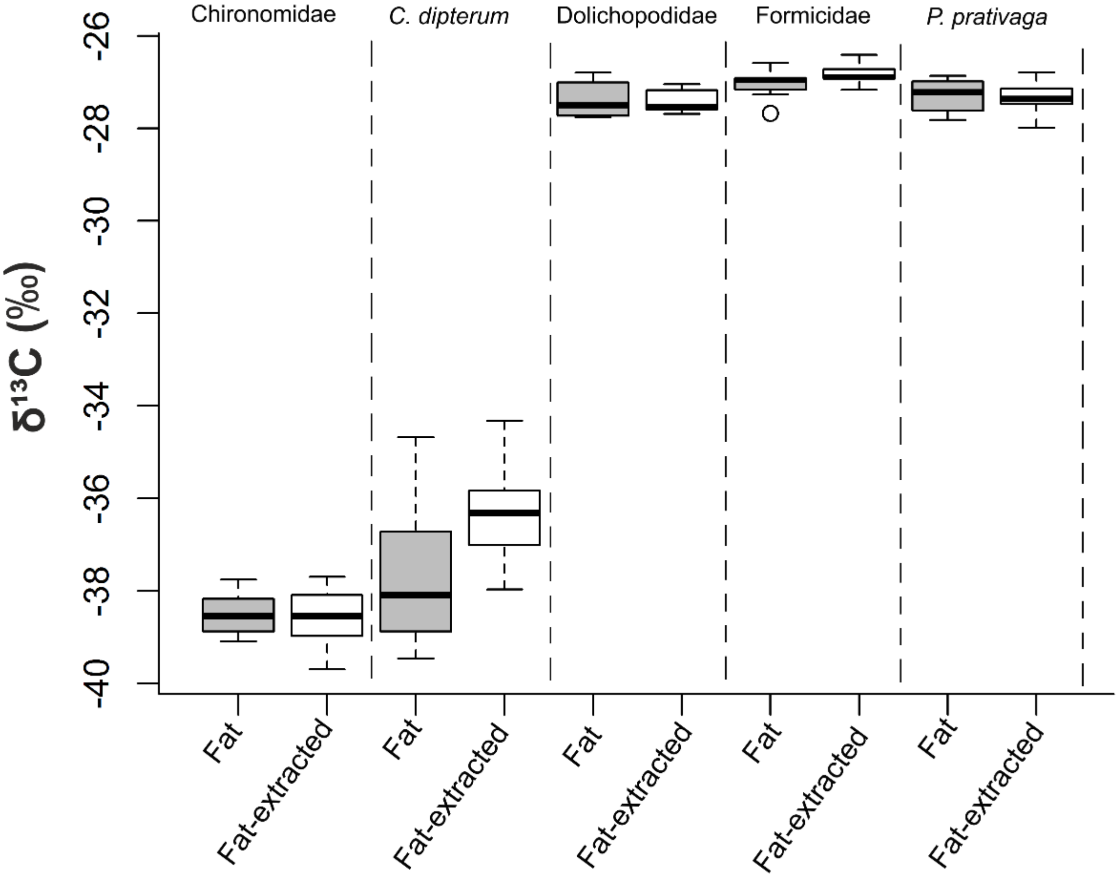
		Longitarsus	sp.
	Dytiscidae	*Ilybius*	sp.
	Hydrophilidae	*Cercyon*	sp.
		Helophorus	sp.
	Leiodidae		
	Pselaphidae		
	Ptiliidae		
	Silphidae	*Silpha*	*obscura*
	Staphylinidae		
Collembola			
Diptera	Ceratopogonidae		
	Phoridae		
Gastropoda			
Hemiptera			
Heteroptera			
Hymenoptera	Braconidae		
	Diapriidae		
	Formicidae	*Lasius*	sp.
		Myrmica	sp.
	Mymaridae		
	Proctotrupidae		
Lepidoptera			
Opiliones	Phalangiidae	*Mitopus*	*morio*
		Nelima	*sempronii*
		Oligolophus	*hanseni*
			tridens
		Phalangium	*opilio*
		Platybunus	sp.
		Rilaena	*trianglularis*
Orthoptera			
Siphonaptera		*Spilopsyllus*	*cumiculi*
Sternorrhyncha		*Aphididae*	sp.
		Drepanosiphidae	sp.
Thysanoptera		*Aeolothripidae*	sp.

Appendix S9. The percentage of aquatic compared to terrestrial flying insects (% flying aquatic) caught at night in the air eclector traps is compared between control and treatment sites prior to ALAN addition (unlit 2012) and during experimental illumination in 2012 (lit 2012) (Comparison I) and in 2013 (lit 2013) (Comparison II) in a BACI design (a). Significant pairwise contrasts are shown for comparison II (solid line; E, F; Fig. 2). Each box plot shows the median, lower, and upper quartiles; greatest and least values excluding outliers (whiskers). The lower panel (b) depict temporal patterns of percentage of aquatic compared to terrestrial per month from May until October 2013 for the treatment and control site. Asterisks are used to indicate significant difference in the pairwise comparisons (*** = p<0.001; ** = p<0.01; * = p<0.05).

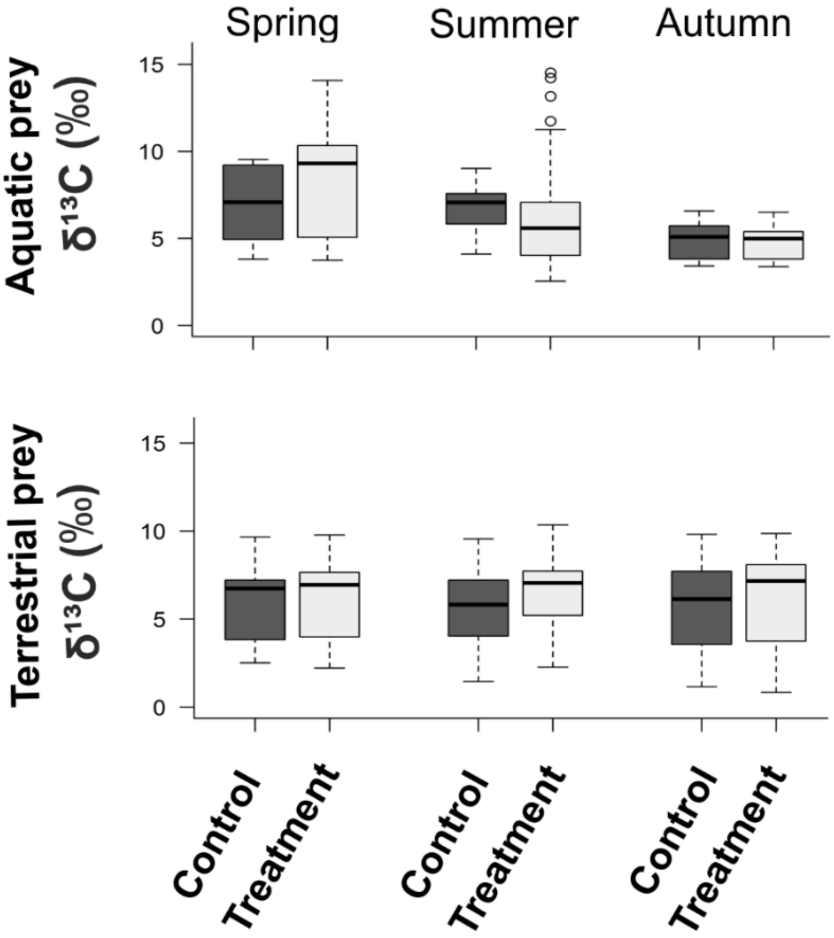


Chapter 4

Appendix S10. Comparison showing no significant difference in δ^{13}C values for samples in which lipids were extracted with samples in which were not (t-test significance>0.05). 10 individuals for each of the 5 selected taxa and condition were analysed. Lipids were removed by Soxhlet extraction using a chloroform/methanol 2:1 solution and a Soxtherm Type SE406 (C. Gerhardt GmbH and Co. KG, Königswinter, Germany). Box plots depict the 25, 50 and 75 percentiles, and whiskers the greatest and least values excluding outliers.



Appendix S11. Comparison of δ^{15}N values between control and treatment sites is depicted for aquatic and terrestrial prey. Box plots depict the 25, 50 and 75 percentiles, and whiskers the greatest and least values excluding outliers. Results are shown for the control and treatment site across the three seasons in 2013.



Appendix S12. Carbon stable isotope ratios ($\delta^{13}C$) (Mean ± SD) and number of samples analysed for each prey and consumer taxon in the control and treatment site across the three seasons in 2013.

Group	Species	Season	Site	N	$\delta^{13}C$ (‰) Mean ± SD
Aquatic prey	Chironomidae	Spring	Control	4	-32.56 ± 1.05
			Treatment	4	-35.50 ± 0.62
	Cloeon dipterum		Control	10	-33.99 ± 1.24
			Treatment	20	-33.93 ± 0.97
	Chironomidae	Summer	Control	10	-36.82 ± 3.10
			Treatment	7	-37.55 ± 1.06
	Hydrophilidae		Control	5	-30.15 ± 3.58
			Treatment	20	-31.80 ± 1.57
	Cloeon dipterum		Control	0	_
			Treatment	12	-34.91 ± 2.13
	Chironomidae	Autumn	Control	5	-35.34 ± 1.40
			Treatment	9	-34.92 ± 1.22
	Cloeon dipterum		Control	10	-34.67 ± 0.19
			Treatment	10	-34.80 ± 0.36
	Erythromma najas		Control	10	-34.42 ± 0.23
			Treatment	10	-34.20 ± 0.22
	Limnephilus binotatus		Control	10	-32.14 ± 0.11
			Treatment	10	-32.11 ± 0.05
Terrestrial prey	Auchenorrhyncha	Spring	Control	5	-27.83 ± 1.20
			Treatment	10	-27.45 ± 1.37
	Chrysomelidae		Control	6	-28.51 ± 0.16
			Treatment	8	-27.90 ± 0.62
	Formicidae		Control	10	-26.55 ± 0.39
			Treatment	20	-26.19 ± 0.54
	Linyphiidae		Control	12	-26.36 ± 0.42
			Treatment	18	-26.46 ± 0.54
	Auchenorrhyncha	Summer	Control	20	-26.85 ± 0.92
			Treatment	20	-26.37 ± 1.88
	Formicidae		Control	15	-26.29 ± 0.57
			Treatment	20	-26.29 ± 0.62
	Linyphiidae		Control	15	-26.86 ± 0.66
			Treatment	20	-26.22 ± 0.59
	Stenorrhyncha		Control	6	-26.56 ± 0.91
			Treatment	5	-27.13 ± 1.19
	Auchenorrhyncha	Autumn	Control	20	-26.26 ± 1.62
			Treatment	20	-26.92 ± 1.77
	Formicidae		Control	20	-26.53 ± 0.48
			Treatment	20	-26.28 ± 0.65
	Linyphiidae		Control	20	-26.14 ± 0.72
			Treatment	20	-25.86 ± 0.48
	Stenorrhyncha		Control	20	-25.26 ± 1.37

			Treatment	20	-25.29 ± 2.05
Consumers	*Pachygnatha clercki*	Spring	Control	20	-28.89 ± 0.26
			Treatment	20	-28.62 ± 0.46
		Summer	Control	18	-28.54 ± 0.64
			Treatment	18	-29.19 ± 0.75
		Autumn	Control	20	-28.59 ± 0.80
			Treatment	20	-28.69 ± 0.75
	Pardosa prativaga	Spring	Control	20	-29.04 ± 0.52
			Treatment	20	-28.36 ± 0.53
		Summer	Control	20	-28.91 ± 0.58
			Treatment	20	-28.42 ± 0.43
		Autumn	Control	20	-28.50 ± 0.80
			Treatment	20	-28.24 ± 0.71
	Rilaena triangularis	Spring	Control	10	-29.09 ± 0.82
			Treatment	10	-28.72 ± 0.24
	Nelima sempronii/ Phalangium opilio	Summer	Control	9	-28.47 ± 0.44
			Treatment	10	-28.12 ± 0.29
	Nelima sempronii	Autumn	Control	10	-28.36 ± 0.63
			Treatment	8	-28.06 ± 0.47

Appendix S13. Bayesian mixing model statistics obtained from SIAR for the relative contribution of aquatic and terrestrial prey to the diet of the analysed consumers for both control and treatment site across the three seasons in 2013. Contribution values are shown as mode and mean and 95% credibility intervals are given.

Consumers	Season	Site	Prey	Min (95%)	Mode	Mean	Max (95%)
Pachygnatha clercki	Spring	Control	Terr	0.66	0.70	0.71	0.77
			Aqua	0.23	0.30	0.29	0.34
		Treatment	Terr	0.69	0.74	0.74	0.79
			Aqua	0.21	0.26	0.26	0.31
	Summer	Control	Terr	0.73	0.79	0.79	0.84
			Aqua	0.16	0.21	0.21	0.27
		Treatment	Terr	0.57	0.66	0.65	0.73
			Aqua	0.27	0.34	0.35	0.43
	Autumn	Control	Terr	0.61	0.67	0.66	0.72
			Aqua	0.28	0.33	0.34	0.39
		Treatment	Terr	0.61	0.67	0.67	0.74
			Aqua	0.26	0.33	0.33	0.39
Pardosa prativaga	Spring	Control	Terr	0.63	0.68	0.69	0.75
			Aqua	0.25	0.32	0.31	0.37
		Treatment	Terr	0.72	0.77	0.77	0.82
			Aqua	0.18	0.23	0.23	0.28
	Summer	Control	Terr	0.69	0.76	0.75	0.81
			Aqua	0.19	0.24	0.25	0.31
		Treatment	Terr	0.69	0.77	0.76	0.83
			Aqua	0.17	0.23	0.24	0.31
	Autumn	Control	Terr	0.62	0.67	0.68	0.73
			Aqua	0.27	0.33	0.32	0.38
		Treatment	Terr	0.66	0.72	0.73	0.79
			Aqua	0.21	0.28	0.27	0.34
Opiliones	Spring	Control	Terr	0.58	0.68	0.68	0.77
			Aqua	0.23	0.32	0.32	0.42
		Treatment	Terr	0.65	0.72	0.72	0.79
			Aqua	0.21	0.28	0.28	0.35
	Summer	Control	Terr	0.70	0.80	0.79	0.88
			Aqua	0.12	0.20	0.21	0.30
		Treatment	Terr	0.69	0.80	0.80	0.90
			Aqua	0.10	0.20	0.20	0.31
	Autumn	Control	Terr	0.61	0.68	0.69	0.77
			Aqua	0.23	0.32	0.31	0.39
		Treatment	Terr	0.63	0.75	0.74	0.86
			Aqua	0.14	0.25	0.26	0.37

Appendix S14. Total number of individuals caught per hour of trap operation (CPUE; catch per unit effort) for aquatic and terrestrial adult flying insects and proportion (%) of aquatic compared to terrestrial insects collected in the air eclector traps at the control and treatment site across the three seasons in 2013 (see Chapter 3).

Sites	Season	CPUE aquatic	CPUE terrestrial	% aquatic
Control	Spring	24.45	33.63	42
	Summer	22.02	121.17	15
	Fall	23.28	54.14	30
Treatment	Spring	270.67	87.50	76
	Summer	6813.08	1042.13	87
	Fall	52.86	76.27	41

Statement of academic integrity

I hereby certify that the submitted thesis “Effect of artificial light at night (ALAN) on interactions between aquatic and terrestrial ecosystems” is my own work, and that all published or other sources of material consulted in its preparation have been indicated. Where any collaboration has taken place with other researchers, I have clearly stated my own personal share in the investigation. I confirm that this work, in the same or a similar form, has not been submitted to any other university or examining body for a comparable academic award.

Berlin, ……………………

....

Alessandro Manfrin