Artificial illumination near rivers may alter bat-insect trophic interactions☆

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ABSTRACT

Artificial illumination at night represents an increasingly concerning threat to ecosystems worldwide, altering persistence, behaviour, physiology and fitness of many organisms and their mutual interactions, in the long-term affecting ecosystem functioning. Bats are very sensitive to artificial light at night because they are obligate nocturnal and feed on insects which are often also responsive to lights. Here we tested the effects of LED lighting on prey-predator interactions at riverine ecosystems, using bats and their insect prey as models, and compared bat and insect reactions in terms of bat activity and prey insect abundance and diversity, respectively, on artificially lit vs. unlit nights. Artificial light influenced both insect and bat assemblages in taxon-specific directions: insect abundances increased at lit sites, particularly due to an increase in small dipterans near the light source. Composition of insect assemblages also differed significantly between lit and unlit sites. Total bat activity declined at lit sites, but this change was mainly due to the response of the most abundant species, *Myotis daubentonii*, while opportunistic species showed no reaction or even an opposite pattern (*Pipistrellus kuhlii*). We show that artificial lighting along rivers may affect trophic interactions between bats and insects, resulting in a profound alteration of community structure and dynamics.

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1. Introduction

Many organisms regulate their activity or life cycles according to light availability over different temporal scales (from diurnal to seasonal), so altering the natural light-darkness turnover has clear implications for several key aspects of their lives (Gaston et al., 2013; Bennie et al., 2016). Nocturnal animals are especially responsive to artificial lighting at night (hereafter ALAN) because they either escape light, as observed in most bat species (Stone et al., 2015; Rowse et al., 2016), or may be lured to it, as happens to many night-active species of insects (e.g. van Langevelde et al., 2011). Nocturnality in bats may have evolved to avoid predation by diurnal avian predators (Rydell and Speakman, 1995), so when

exposed to ALAN, bats may face, or at least perceive, an increased risk of predation (Stone et al., 2015). Either way, most bat species are intolerant to ALAN, whose occurrence may in fact exclude them from roosting (Rydell et al., 2017), foraging (Stone et al., 2012; Lewanzik and Voigt, 2014) and drinking (Russo et al., 2017, 2018) sites, or severe their commuting routes (Stone et al., 2009), with negative effects on their survival and reproduction success.

Over two thirds of living bats are obligate or facultative insectivores (Kunz et al., 2011), so their survival is strictly linked with insect food availability. Many of their prey also exhibit sensitivity to ALAN (Eisenbeis, 2006), so besides affecting bats directly, lighting may also interfere with bat activity indirectly by altering foraging success. While most bat species avoid lit foraging sites, however, a handful of opportunistic bat species brave artificial lighting to forage near street lamps and capture arthropods that are lured to the light (e.g. Rydell and Racey, 1995), which brings about important ecological (e.g. Stone et al., 2015) and perhaps evolutionary (Tomassini et al., 2014) implications. Moths attracted to roadside

lighting from adjoining naturally dark habitats may become easy prey to such opportunistic bats and thus decline in their original habitat, to which light-averse bat species, which never venture into lit areas, are restricted (Arlettaz et al., 2000). This "vacuum effect" (Stone et al., 2015) may impoverish or deplete prey availability in natural habitats, implying complex, detrimental alterations of prey-predator dynamics.

Freshwater ecosystems are home to a great diversity of insects with aquatic larval stages (Müller, 1982) whose flying adults represent a primary food source for riparian predators (Baxter et al., 2005), among which the many bat species that forage over water or near riparian vegetation (e.g. Fukui et al., 2006). Such bats include the so-called "trawling bats", which are specialized in foraging over water (Nardone et al., 2015), typically scooping out prey from its surface with their feet or wing membrane (Siemers et al., 2001).

As in other ecosystems, ALAN may have adverse impacts on arthropod communities also in freshwater sites (Manfrin et al., 2017): insects may experience an increase in mortality getting exhausted from their own strong phototactic reaction, or by concentrating near lights where they are exposed to unusually high predation (Eisenbeis, 2006; Szaz et al., 2015). Effects on emerging insects span through different biological scales, from that of individuals – such as reductions in body size or disruption of dispersal patterns (Horváth et al., 2009; Meyer and Sullivan, 2013; Perkin et al., 2014) – to that of communities, including declines in taxonomic richness (Meyer and Sullivan, 2013).

The effects of ALAN on freshwater ecosystems may be so pervasive to alter the complex food webs that link aquatic insects with riparian predators, as seen for arthropods assemblages (Manfrin et al., 2017). Whether such alterations also regard the trophic relationships between foraging bats and their insect prey is unknown. Given the crucial role freshwater ecosystems play for both bats and insects, and the strong trophic relationship existing between these two faunal components, it is highly likely that ALAN will interfere with prey-predator dynamics through both direct and indirect effects, yet no study has so far addressed this potentially important process. The magnitude of this phenomenon is probably considerable since ALAN very often occurs near rivers or along lakeshores in urbanized areas for safety or aesthetic reasons (Kummu et al., 2011), and high densities of streetlights are usually recorded near freshwaters (Manfrin et al., 2017).

To tackle this issue, we carried out paired comparisons of bat activity and insect abundance at bat foraging sites along two rivers under experimentally lit vs. naturally dark conditions. By assessing insect food availability under such conditions, we could disentangle direct (phototactic) reactions by bats, for instance light avoidance, from indirect (food-driven) reactions, such as an increase (or decrease) in bat activity in response to any light-dependent insect food increase (or decline). On such counts, we formulated the following hypotheses and predictions:

1) ALAN is known to affect bats, in most cases adversely (Stone et al., 2015), so we hypothesize that negative reactions will occur when sites are illuminated. We therefore predict a decline in general bat activity under such conditions;

2) As seen in other habitat types, in rivers too, we hypothesize that lighting will affect different species in different ways depending on their degree of tolerance to lighting (e.g. Rydell, 1992; Voigt et al., 2018a,b) which in turn depends on their ability to escape predators (Zeale et al., 2016). Slow-flying species, with short, broad wings such as *Myotis* spp. and *Plecotus* spp., are more manoeuvrable but also potentially more exposed to predation than fast-flying species, characterized by longer and narrower wings (Svensson and Rydell, 1998; Stone et al., 2012; Spoelstra

et al., 2017a,b; Russo et al., 2018). Therefore, we predict a decline in the activity of the former, whereas activity of faster fliers is predicted to either remain unchanged or increase under lit conditions;

3) We hypothesize that ALAN will alter insect abundance across the river, predicting that lighting will lure photopositive insects from the water surface, as well as from the adjacent riparian vegetation, towards the lit bankside, which will imply a decline in insect abundance over water and an increase in insect abundance on the lit bank.

4) As insect taxa show different reactions to ALAN, this may disrupt their community structure and dynamics (Owens and Lewis, 2018), so we also hypothesize that the composition of insect assemblages under lit and dark conditions will differ, and predict an increase in the presence of positive phototactic or diurnal species when the light is switched on.

2. Materials and methods

2.1. Study area

We did our work in a mountainous area (840–1093 m a.s.l.) of the Central Italian Apennines, at the Abruzzo Lazio and Molise National Park (41°47′20″ N; 13°46′33″ E) and in its buffer zone, along two watercourses, the Sangro River and its tributary stream Zittola. The area is mainly covered with broadleaved forest dominated by *Fagus sylvatica* and, at lower elevations, *Quercus* spp., interspersed with pastures and small farmland patches. Villages also occur along the course of the Sangro River, but all experimental sites were far enough from urbanized areas to avoid interferences with their artificial lighting. In most cases, riparian vegetation (mostly characterized by *Salix alba* and shrubs) is well developed along the watercourses.

2.2. Study design and experimental protocol

To test the effects of ALAN on bats and insects, we adopted a paired design: at each site, we assessed bat activity and estimated insect abundance respectively under dark (control) and artificially lit (treatment) conditions. To illuminate sites, we used a portable LED outdoor lamp (McMantom, Italy) made of 48 high-power LEDs that generated a light intensity of 6480 lm (4000–4500 K) at 25 °C with a power rating of 32 ± 2 W. We powered the light with a 12 V (35A) car battery switched on manually. The lamp's light spectrum had a bimodal pattern typical of LED lighting with two peaks of relative luminous flux at 450 nm and 590 nm (data provided by the producer). We checked that the system emitted neither audible nor ultrasonic noise so that light was the only factor potentially influencing bat and insect behaviour. We placed the light unit on a pole at 3 m above the ground and ca. 1 m from the river bank, and made sure it illuminated the whole cross section of the water course (on average, ca. 7 m), corresponding to a mean illuminance of 250–310 lux across the lit area. We switched the light on at sunset and off ca. 3 h later.

At each site, treatment nights always followed dark ones to avoid carryover effects of lighting that might have influenced bat behaviour in the control nights. Similarly, bat and insect sampling sessions took place on different nights to avoid any interference between the traps and bat activity. Bats might show lunar phobia due to an increase in perceived predation risk (Saldaña-Vázquez and Munguía-Rosas, 2013), so we took moon phase into account as a potential factor influencing bat activity. On each night, we recorded moon phase as the percentage of visible lunar disk

(retrieved from http://nautica.it; Russo and Jones, 2003). Moon was recorded as "absent" when moonrise occurred at least 30 min after a sampling session was over, or moonset took place at least 30 min before a sampling session began.

2.3. Bat and insect sampling

Fieldwork took place between June and July 2017 along the two above-mentioned water courses at eight bat foraging sites identified during previous bat detector surveys and radiotracking work (Sangro River: n = 7; Zittola Stream: n = 1; Nardone et al., 2015; De Conno et al., 2018). To ensure independence, sites were all > 2 km apart from each other. We worked on nights with little or no cloud cover, rain, and wind, and with minimum temperatures >10 °C. Since ripples may reduce bat foraging efficiency (Frenckell and Barclay, 1987; Mackey and Barclay, 1989; Rydell et al., 1999), we made sure that at all sites water turbulence was absent or limited. We surveyed bat activity at each sampling point with stationary, automatically triggered D500X bat detectors (Pettersson Elektronik, Uppsala, Sweden). The D500X remotely records the ultrasonic spectrum up to 190 kHz, covering the entire frequency range of all bat species potentially encountered in Italy, up to the ca. 110 kHz of *Rhinolophus hipposideros* (Russo and Jones, 2002). At each site, we recorded bats for one night, placing the D500X on the riverside, ca. 2 m from the light pole, as close as possible to the water, and pointing it upwards at 45°. At all recording sites, riparian vegetation did not obstruct bat flight paths over the water surface. We used the following recording settings for the both sampling sessions (dark and lit nights): 500 kHz sampling rate; 5 s recording duration from trigger; 60 s of recording pause after each record; high pass filter enabled at 10 kHz; and low trigger sensitivity, to avoid recording background noise. We saved recordings on compact flash cards as WAV files. We first screened recordings visually in BatSound 4.1; then, we generated oscillograms, power spectra and spectrograms to measure call variables following the measurements shown in Russo and Jones (2002), and using a 1024-pt FFT Hamming window with a 98% window overlap. We identified species using echolocation calls whose frequencies, duration and frequency vs. time course allowed safe identification, or social calls, when these were diagnostic (e.g. Pfalzer and Kusch, 2003; Russo et al., 2009; Nardone et al., 2017). We could exclude the presence of *M. capaccinii*, whose calls are similar to those of *Myotis daubentonii* (widespread in the study area; e.g. Nardone et al., 2015), as the former was never observed in 20 years of mistnetting in the area (D. Russo, unpublished data). When call structure or recording quality did not meet the standards needed for detailed species discrimination, we classified calls to genera, which happened, respectively, for unidentified *Myotis* and *Nyctalus*. We pooled together recordings into six 30-min intervals, and quantified bat activity as the number of bat passes (Russo and Jones, 2003) which occurred during each interval. Since bat passes are less likely to be missed than feeding buzzes in recordings, we assumed the former to be a proxy for bat foraging activity, which is justified by the strong positive correlation existing between the two variables (for *M. daubentonii*, dominant in our sample, r = 0.75, p < 0.001).

We sampled insects at all sites surveyed for bats using four sticky traps per nights, each made of a round yellow plastic plate (diameter: 22 cm) sprayed on both sides with glue (Vebi Istituto Biochimico srl.). At each site, we deployed one plate onshore hung to the light pole (trap 1), and three plates above the water, hung to a horizontal nylon wire stretched between the river shores at ca. 10 cm from the water surface, i.e. two at 1 m from each bank (traps 2 and 4) and one at the centre of the riverbed (trap 3). In this way, traps were > 2 m apart from each other, and at increasing distances from the light pole, from 1 to ca. 7 m (Fig. A2 in Online Appendix).

We left traps in place for 3 h after sunset, then we brought them to the laboratory for insect identification and abundance estimation. Due to the very high numbers of trapped insects, we refrained from counting them exhaustively: instead, since insects were evenly scattered across the plates, we estimated abundance by superimposing a 3×3 cm square cell grid covering the entire plate and counted and identified all those present in 10 randomly selected cells per plate (5 cells per face). We used this information to work out the mean number of insects of each identified taxon/cm^2 and extrapolated this value to the entire plate surface. Insects that occurred between two cells were conventionally assigned to the cell containing the insect head. We focused our analysis on dipterans, as this order makes for the most abundant bat prey in rivers (e.g. Nardone et al., 2015). We identified all insects at least to the order level, and dipterans to the highest taxonomical level possible (family or suborder) by inspecting visually their diagnostic features with an Olympus SZX12 stereomicroscope using published keys (Oosterbroek, 2006) and reference material.

2.4. Statistical analyses

To test for the effect of ALAN on bat activity, we applied Linear Mixed Models (LMMs) with a binomial negative distribution and a log-link function using the lme4 package (Bates, 2010) in R 3.3.2 (R Core Team, 2016). We analysed only species for which we recorded >30 bat passes. We built models including either total or single-species bat activity as the response variable, experimental condition (treatment vs control) as the explaining variable, percent of visible moon as a covariate, and site as a crossed random factor. We evaluated model fit by checking R^2 values, and assessed the direction and magnitude effects on bat activity for each model by checking variable estimates and standard errors, setting significance at p < 0.05.

We tested differences in insect abundance under dark and lit conditions by adopting paired Student t-tests, in which plates placed in the same position at each site were paired under the two conditions. We ran the tests separately at family, suborder, order and total sample levels, limiting the analyses to those groups for which we caught at least 30 individuals, and considered results significant when p < 0.05. To look at possible changes in insect assemblage composition at family level between dark and lit conditions, we also calculated a Bray-Curtis dissimilarity index (Bray and Curtis, 1957) for all samples; this index ranges between 0 (all taxa shared between conditions) and 1 (no taxon in common between them). To examine insect assemblage composition among sites and conditions we used non-metric multidimensional scaling (NMDS). This technique ordinates samples according to their dissimilarity based on a distance matrix; the latter is obtained from the difference in insect composition and experimental variables (site, treatment, and trap position) among sites. To test whether insect assemblages differed under different light conditions, sites and trap positions, we conducted a two-way analysis of similarity (ANOSIM, Clarke, 1993). ANOSIM uses a bootstrap randomization (9999 replicates) to calculate the probability of group membership based on variances (between- and within-sample) in the community. Finally, to quantify the relative importance of each environmental variable in grouping sampling sites we calculated the correlation coefficients between each variable and the NMDS scores. Both NMDS and ANOSIM are based on rank distances between samples (we used Bray-Curtis dissimilarity and Euclidean distances). An analysis of similarity percentage (SIMPER) was also conducted to rank insect taxa according to their contribution in affecting sample grouping. We ran NMDS, ANOSIM and SIMPER analyses in PAST 3.22 (Hammer et al., 2001; https://folk.uio.no/ohammer/past/).

3. Results

3.1. Bat activity

We recorded 2,489 bat passes from 8 species and two species groups. The most frequently recorded species was *M. daubentonii*, followed by *Pipistrellus kuhlii*, *Hypsugo savii* and *P. pipistrellus* (Table A1 in Online Appendix).

In agreement with our first hypothesis, total bat activity was significantly influenced by ALAN (Table 1), showing a significant decrease under lit conditions, and also declined later at night. The analyses done at species level showed that the direction of bat response to ALAN was species-specific, in agreement with our second hypothesis. Namely, while *M. daubentonii* activity declined significantly under lit conditions (22.3 ± 20.2 and 16.8 ± 12.5 passes per interval, respectively) and later at night, *P. kuhlii* had an opposite reaction (1.3 ± 2.1 and 1.8 ± 2.4 passes per interval, respectively), i.e. the latter species slightly yet significantly increased activity on lit sessions and did not show changes in the course of the night (Table 1). We detected no significant effect for the other species or species-groups that we analysed (those for which we had > 30 bat passes), i.e. *H. savii*, *P. pipistrellus*, *Nyctalus* spp., and unidentified *Myotis* (Table A2 in Online Appendix).

3.1.1. Insect abundance and diversity

We estimated a total number of 21,240 sampled insects (Table A3 in Online Appendix), and, as we hypothesized, both the total number of sampled insects and that of total dipterans were significantly higher under lit conditions at the two plates closer to the light, yet no difference was found under dark vs. lit conditions at the remaining traps farther away from the lamp. The same response was also observed for nematocerans, and for three families of this group, respectively Psychodidae (only at the plate closest to light), Ceratopogonidae and Chironomidae (Table 2; Table A4 in Online Appendix). Dark and lit conditions shared a relatively small number of insect taxa, as they resulted in a Bray-Curtis dissimilarity index value of 0.94. Under lit conditions, in particular, we trapped six insect groups that we never recorded under dark conditions (Hymenoptera, Neuroptera and four Brachyceran families), while only three groups were caught under dark but not lit conditions (one Brachyceran and one Nematoceran family, and Ephemeroptera).

We found significant differences in the composition of insect assemblages (NMDS stress = 0.13; ANOSIM: R = 0.14, p < 0.001; Table A5; Fig. A3 in Online Appendix), and, as hypothesized, ALAN had a major effect on it (Table A5 in Online Appendix). Insect samples were first grouped according to treatment (light vs. dark, R = 0.17, p < 0.001), which had the greatest influence, then by trap position (R = 0.15, p < 0.001), while site had no significant effect

(R = 0.09, n.s.). According to SIMPER results (Table 3) the top three taxa contributing to dissimilarity among samples were Chironomidae (34.07%), Hemiptera (12.70%) and Ceratopogonidae (11.39%).

4. Discussion

In accordance with our first hypothesis, total bat activity declined in response to experimental illumination of river stretches, as also shown by previous studies that recorded adverse effects of ALAN on bats (Mathews et al., 2015; Stone et al., 2015; Rowse et al., 2016).

The response that total bat activity showed was mostly driven by one, dominant species, the trawling bat *M. daubentonii*, which was much more frequent than any other bat species we recorded, accounting for almost 80% of total bat passes. When activity was analysed at species (or species group) level, we found that, as expected, reactions were species-specific: *M. daubentonii* decreased activity in response to ALAN, while opportunistic species (a minority at all sites) that are known to tolerate lighting either kept their activity unchanged on lit relative to dark recording sessions, or even increased it under the former condition. Changes in food availability fail to explain the decline recorded in *M. daubentonii* on lit sessions, because, overall, Chironomidae and Ceratopogonidae boosted in numbers under such conditions, and although the increase mostly took place in the space near the lamp, i.e. closer to the riparian vegetation, the insect community over the water, where these bats mostly hunt, showed at least no significant qualitative or quantitative changes. Overall, the insects that were available (and abundant) under lit conditions are typical prey of *M. daubentonii* (e.g. Racey et al., 1998; Nardone et al., 2015; Todd and Waters, 2017), and may account for >95% of this species' diet (Vaughan, 1997), so they should have sustained its foraging activity. This implies that the decline in activity we observed in this species is best explained as direct avoidance of artificial lighting rather than a food-induced reaction. We cannot rule out that some of the *M. daubentonii* passes recorded were associated to activity outside the light cone, in which case activity in the lit area would have been even lower than that we estimated.

A relatively slow flier, *M. daubentonii* was expected to reduce activity in response to ALAN according to our hypothesis. *M. daubentonii* avoids its preferred habitat in the Nordic midsummer nights, when natural illumination is strong (Nyholm, 1965), and is deemed as one of the most light-averse bat species (Voigt et al., 2018a,b), as much as lighting of waterways is seen as a serious threat to it. Yet, recent work on this species that tested the effects of light of different colour on individual *M. daubentonii* passing through culverts underneath a road showed no reaction to lighting, regardless of its colour (Spoelstra et al., 2018). It is important to remark, however, that the experimental set used by Spoelstra et al. (2018) was very different from ours because in that study the bats were commuting, while in our case we tested the effect of ALAN on foraging bats, which might be more strongly light-averse than commuting individuals due to their longer exposure to illumination which makes them more vulnerable to predation. *M. daubentonii* mostly move along rivers when hunting, rarely venturing out of their course (e.g. Nardone et al., 2015), so ALAN may act as a barrier fragmenting this species' foraging habitat.

Based on current knowledge, the light-exploiting bat species basically belong to two categories, which are quite different from each other from an ecological viewpoint. Some of these species may cross, or even forage at illuminated sites thanks to their fast flight, which renders them efficient at escaping predators, decreasing the actual or perceived predation risk under lit conditions (Mathews et al., 2015). This is the case with *Nyctalus* spp., which in our

Table 1
Effects of artificial light (Treatment), time (Session) and moonlight (Moon phase) on bat activity over river sites (n = 8) in the Abruzzo Lazio and Molise National Park, Italy. ***: p < 0.001; **: p < 0.01; *:p < 0.05; n.s.: not significant. Only cases featuring significant effects are illustrated.

Model	R^2	Variable	Estimate±SE	Z	P
Total activity	0.43	Treatment	-0.39 ± 0.12	12.63	**
		Session	-0.09 ± 0.04	31.69	*
		Moon phase	-0.00 ± 0.00	10.54	n.s.
Myotis daubentonii	0.64	Treatment	-0.54 ± 0.39	27.44	***
		Session	-0.14 ± 0.03	27.68	***
		Moon phase	-0.00 ± 0.00	12.38	n.s.
Pipistrellus kuhlii	0.52	Treatment	0.18 ± 0.11	4.75	*
		Session	-0.03 ± 0.04	28.92	n.s.
		Moon phase	-0.00 ± 0.00	0.13	n.s.

Table 2
Comparison of insect abundance (mean ± standard deviation) estimated respectively under dark (Dark) and artificially lit (Light) conditions at eight river sites in the Central Italy Apennines. Trap position indicates the location of each plate relative to the artificial light source and river transversal section (see text). ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; n.s. = not significant; NA: not applicable. Only taxonomic groups that showed significant results are presented.

Group	Trap position	Light	Dark	T	P
Total insect abundance	1	6.99 ± 1.73	2.24 ± 1.00	−5.93	0.0005 ***
	2	4.31 ± 0.88	2.43 ± 1.54	−3.20	0.01 *
	3	3.81 ± 1.30	3.65 ± 1.00	−0.50	n.s.
	4	2.70 ± 1.17	2.16 ± 1.42	−0.93	n.s.
Diptera	1	22.44 ± 14.41	2.04 ± 2.00	−3.71	0.008 **
	2	6.41 ± 2.98	3.36 ± 2.46	−2.50	0.04 *
	3	5.00 ± 3.26	6.86 ± 4.83	1.38	n.s.
	4	3.72 ± 2.27	3.23 ± 3.24	−0.36	n.s.
Nematocera	1	19.00 ± 11.41	1.68 ± 1.58	−3.96	0.005 **
	2	5.58 ± 1.20	2.04 ± 2.33	−3.05	0.01 *
	3	4.09 ± 1.70	4.82 ± 3.09	0.73	n.s.
	4	3.16 ± 1.73	2.04 ± 2.00	−1.04	n.s.
Chironomidae	1	6.61 ± 1.69	0.28 ± 0.79	−9.51	2.978e-05 ***
	2	4.18 ± 0.85	0.36 ± 1.02	−8.89	4.614e-05 ***
	3	3.25 ± 1.88	2.51 ± 1.27	−2.29	0.04 *
	4	1.41 ± 1.53	1.20 ± 1.30	−0.29	n.s.
Ceratopogonidae	1	3.71 ± 2.92	0.28 ± 0.79	−3.53	0.009 **
	2	0	0	NA	NA
	3	0	0	NA	NA
	4	0	0	NA	NA
Psychodidae	1	1.71 ± 1.91	0	−2.52	0.04 *
	2	0	0.28 ± 0.79	1	n.s.
	3	0	0	NA	NA
	4	0.28 ± 0.79	0.28 ± 0.79	0	n.s.

Table 3
SIMPER percent dissimilarity of insect assemblages at 8 river sites in Central Italy Apennines. Taxa contributing <0.20% are not shown.

Taxon	Average dissimilarity	Contribution %	Cumulative %
Chironomidae	34.07	36.74	36.74
Hemiptera	12.70	13.69	50.43
Ceratopogonidae	11.59	12.49	62.93
Empidideae	9.23	9.95	72.88
Tipulidae	6.93	7.47	80.35
Psychodidae	4.74	5.11	85.46
Lonchopteridae	3.28	3.53	88.99
Sciaridae	3.06	3.30	92.29
Trichoptera	2.93	3.16	95.45
Coleoptera	2.72	2.93	98.39
Cecidomyiidae	1.01	1.09	99.47
Hymenoptera	0.31	0.33	99.80
Lepidoptera	0.80	0.20	100.00

experiments did not change activity from unlit to artificially lit nights. Other species are adapted to emerge soon after dusk or sometimes even before sunset to exploit their staple food, which is given by small crepuscular insects, mostly dipterans (Goiti et al., 2003; Tomassini et al., 2014; Russo & Ancillotto, 2015; Ancillotto et al., 2016). Pipistrelle bats (*P. kuhlii, P. pipistrellus, H. savii*) belong to the latter category (e.g. Haffner and Stutz, 1985; Tomassini et al., 2014), which explains why in our study their activity did not decline or (in *P. kuhlii*) even increased under lit conditions. The increase in *P. kuhlii* activity we observed under ALAN was most likely due to the increase in small dipterans which concentrated especially near the light, since the latter had the effect of promoting prey availability along riparian vegetation, the optimal foraging habitat for this edge specialist (Ancillotto et al., 2016). Insect groups that were attracted by light in our experiment account for ca. a third of *P. kuhlii* diet (Goiti et al., 2003), and tympanate moths that normally react to bat calls avoiding attacks become more accessible when lights are on because ALAN partly impairs their evasive manoeuvres (e.g. Svensson and Rydell, 1998; Acharya and Fenton, 1999; Wakefield et al., 2015). The rapid

response by *P. kuhlii* to an unpredictable food source such as insects concentrating near new artificial lights confirms sensitivity of bats to transient habitat changes (Bell, 1980). More permanent lighting might also act as conspicuous landmarks of foraging sites for *P. kuhlii* and other light-tolerant species, but due to conservation reasons, we could not test it.

In our experiment, we used white LED lighting, but different light spectra might lead to different reactions by both bats (Spoelstra et al., 2017a,b; Voigt et al., 2018a,b) and insects (van Grunsven et al., 2014), so repeating our experiments with lights of different colours would make it possible to see whether responses may change accordingly. For instance, bats seem to tolerate red lights much more than other colours (Spoelstra et al., 2017a,b), and insects are less attracted by light missing the blue component of the spectrum (van Grunsven et al., 2014).

As predicted, when the light was on, we recorded an increase in insect abundance at the traps closer to it, which suggests once more that ALAN attracts photopositive insects from nearby sites exerting a "vacuum effect" on them (Stone et al., 2015). However, in contrast with our prediction, rather than decreasing, insect abundance remained unchanged on lit sessions farther away from the lamp, i.e. along the interbank transect where we placed the remaining traps. We speculate that this lack of change may not reflect a static situation, instead it might be an effect of insects being lured continuously to the lit area from nearby sites where we did not sample insects (in this case, such sites would undergo a reduction in insect abundance). This would keep insect density relatively constant over the water and eventually concentrate insects near the lamp. Alternatively, ALAN might have increased emergence of aquatic insects, as previously documented (Manfrin et al., 2017), compensating for the reduction in insect abundance caused by their positive phototaxy from the middle of the river section towards the lamp.

Some taxa that were not present under dark conditions turned up when the light was on. Namely, as predicted, some groups of positively phototactic or diurnal insects among brachycerans, neuropterans or hymenopterans only appeared under such conditions, while to a more limited extent, other insect groups were only

present under dark conditions. Moreover, even groups that were present under both dark and lit conditions showed compositional changes at a finer-grained taxonomic resolution, as we observed within Chironomidae, Hemiptera and Ceratopogonidae, whose differences under lit vs. dark conditions caused most of the dissimilarity among samples. Noticeably, qualitative changes in insect assemblage composition took place where the two insect traps closest to the lamp were located, matching the pattern we recorded for insect abundance, so the strongest effects on insects are likely to take place along the banks and their immediate surroundings. Once more, the action of ALAN resulted in qualitative and quantitative changes of insect assemblages that may disrupt their community structure and dynamics (Owens and Lewis, 2018).

5. Conclusions

Our is the first contribution towards understanding ALAN-driven alteration of predation dynamics in bat-insect interactions that take place in riparian habitats, a topic thus far unexplored. Because in our experiment ALAN concentrated photopositive insects near the lamp and likely inhibited evasive manoeuvres by tympanate prey, these may have faced an increased predation risk by light-exploiting bat species along the banks, potentially exposing to increased mortality also insect taxa that would otherwise not be present at night in that habitat (Cravens et al., 2018). We did not establish that the diet of the bats under study changed depending on the presence or absence of ALAN, so we cannot fully conclude that ALAN alters trophic relationships between bats and their prey. However, such alteration is highly likely since both predators and prey experienced quantitative and qualitative changes (Rydell, 1992).

While further research is needed to ascertain bat diet and the effect of light colour in such contexts, based on our findings we highlight that due to the massive presence of lighting along the banks of many rivers, for example those crossing urban areas or near roads, the effects of ALAN on such sensitive ecosystems may be widespread and in most cases are likely to imply a profound alteration of trophic relationship between bats and insects, with considerable consequences for ecosystem functioning. Mitigation measures such as part-time lighting may prove ineffective, because this would still largely interfere with insects and their bat predators. The use of "intelligent" automatic illumination that switches on only when necessary (Stone et al., 2015; Russo et al., 2017), or the adoption of full cutoff lighting (Voigt et al., 2018a,b), might partly mitigate the impact of ALAN in such situations, but based on our findings we strongly recommend that artificial lighting near rivers and other waterbodies is avoided unless strictly necessary, since adverse effects might persist even when such mitigation measures are taken.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.06.105.

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