

Experimental tests of light-pollution impacts on nocturnal insect courtship and dispersal

Ariel Firebaugh¹  · Kyle J. Haynes^{1,2}

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Abstract Though a number of effects of artificial light pollution on behavior and physiology have been described, there is little understanding of their consequences for the growth and distribution of populations. Here, we document impacts of light pollution on aspects of firefly population ecology and underlying mating behaviors. Many firefly species have a unique communication system whereby bioluminescent flashes are used in courtship displays to find and attract mates. We performed a series of manipulative field experiments in which we quantified the effects of adding artificial nighttime lighting on abundances and total flashing activity of fireflies, courtship behaviors and mating between tethered females and free-flying males, and dispersal distances of marked individuals. We show that light pollution reduces flashing activities in a dark-active firefly species (*Photuris versicolor*) by 69.69 % and courtship behavior and mating success in a twilight-active species (*Photinus pyralis*). Though courtship behavior and mating success of *Photinus pyralis* was reduced by light pollution, we found no effects of light pollution on male dispersal in this species. Our findings suggest that light pollution is likely to adversely impact firefly populations, and contribute to wider discussions about the ecological consequences of sensory pollution.

Keywords Fireflies · Communication · Sensory ecology · Population ecology · Anthropogenic disturbance

Introduction

Degradation of the sensory environment is a component of habitat loss. As land use intensification alters the structural landscape, the resulting spread of chemical, noise, and light pollution can affect habitat quality because of their impacts on the sensory information organisms use to interpret and interact with their surroundings (Francis and Barber 2013, Slabbekoorn et al. 2010; Longcore and Rich 2004; Scott and Sloman 2004). Artificial light at night erodes nighttime darkness, a potential key component of habitat suitability for many species. Light pollution—defined here as illuminance from human activities—already envelops over 19 % of the global land surface area and the affected area is increasing at a rate of 6 % per year (Cinzano et al. 2001). As light pollution spreads, understanding its ecological consequences is increasingly critical.

Light pollution is known to influence a range of biological activities [e.g., communication and movement (reviewed in Gaston and Bennie 2014; Gaston et al. 2014; Rich and Longcore 2006)]. Many organismal responses to light pollution—including phototaxis causing flying insects to circle lights to the point of exhaustion—are likely to influence demographic rates, in turn leading to impacts on abundance (Perkin et al. 2014; Frank 2006). However, the effects of light pollution at the population level remain unclear (Gaston and Bennie 2014). One problem is that many studies only report relationships between light pollution and species

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✉ Ariel Firebaugh
alf7f@virginia.edu

¹ Department of Environmental Sciences, University of Virginia, Clark Hall, 291 McCormick Road, Charlottesville, VA 22904-4132, USA

² Blandy Experimental Farm, University of Virginia, 400 Blandy Farm Lane, Boyce, VA 22620, USA

distributions or abundances without exploring their underlying causes.

Fireflies are an ideal study system for investigating how light pollution affects populations and the role of behavioral changes under field conditions. Many firefly species are nocturnal and use a visual communication system consisting of bioluminescent flashes to find and attract mates (Lloyd 1971). These courtship activities are sensitive to environmental light conditions. Flashing begins at or sometime after sunset when low ambient light levels enhance the contrast between the flash signal and the background environment (Lall et al. 1980), and the timing of nightly flashing activities can be advanced or delayed by natural variation in light levels due to cloud cover or the phase of the moon (Dreisig 1974; Allard 1931). The importance of light as a cue for firefly flashing has been corroborated by laboratory behavioral studies, which show that exposure to artificial light can stop or delay the timing of firefly flashing (Merritt et al. 2012; Dreisig 1975; Buck 1937; Rau 1932). These findings, combined with the observation that common lighting technologies overlap firefly flashes spectrally (Elvidge et al. 2010; Lall and Worthy 2000), have led to the conjecture that light pollution may disrupt flash communication systems, with cascading effects on mating success and population persistence (Bauer et al. 2013; Faust et al. 2012; Lloyd 2006). Light pollution is considered a conservation threat for firefly populations worldwide (Bauer et al. 2013; Faust et al. 2012; Lloyd 2006); however, the effects of light pollution on populations are poorly understood.

Assuming a count of firefly flashes as a reasonable proxy of abundance, some studies have shown that light pollution affects firefly abundances and distributions. Hagen et al. (2015) observed reduced firefly activity when outside lighting was turned on, and Viviani et al. (2010) reported changes in firefly species composition across a spatial gradient in light levels. Picchi et al. (2013) observed *Luciola italica* fireflies less frequently in more light-polluted areas of Turin, Italy as well as locations surrounded by a higher proportion of urban land cover. These surveys provide valuable observational survey data; however, given that light pollution is likely to increase with urban land cover (Small et al. 2005), experimental studies are needed to isolate effects of light pollution from other urban landscape characteristics.

In this study, we examined the effects of light pollution on the local abundances, dispersal, and mating activity of fireflies to inform inferences about population-level impacts. In one experiment, nighttime light pollution was manipulated in replicated experimental plots in a native plant meadow inhabited by two firefly species (*Photinus pyralis* and *Photuris versicolor*). Within these plots, we recorded counts of firefly flashes (a proxy for abundances) weekly over the course of a summer. In addition, to examine the potential importance of net movement away

(repulsion) or toward (attraction) sources of artificial light, we conducted a mark-release-recapture study with *P. pyralis* males. We also investigated whether light pollution affects the mating behaviors and mating success of *P. pyralis*. We expected light pollution to cause local reductions in abundance due to net movement away (repulsion) from nighttime light sources. We also anticipated light pollution would reduce courtship activities and mating success.

Materials and methods

Study organisms

Two species (*Photinus pyralis* and *Photuris versicolor*) dominate the firefly community at Blandy Experimental Farm (BEF), a University of Virginia environmental research station in the northern Shenandoah Valley of Virginia where this work was conducted (39°03'50.43"N, 78°03'47.20"W). *P. pyralis* is a common firefly species throughout eastern North America. Male *P. pyralis* display a characteristic J-shaped flight path while flashing. Female *P. pyralis* are capable of flight over short distances, but perch on vegetation during nightly courtship activities (Lloyd 1966). *P. pyralis* flash courtship activities usually occur within 90 min of sunset at BEF. *Photuris versicolor* adults are predacious-luring prey firefly species (including *Photinus pyralis*) by mimicking their distinctive flash patterns (Lloyd 1980). At BEF, *P. versicolor* is most active 1–3 h after sunset. *Photuris versicolor* flash patterns occur with a higher frequency than those of *Photinus pyralis* (Lloyd 1990), making it easy to differentiate between the two species based on their flash characteristics. In the following experiments, all applicable institutional and/or national guidelines for the care and use of animals were followed.

Flashing activities and abundances

A manipulative field experiment was conducted to examine the effects of light pollution on firefly flashing activities and abundances. We established eight experimental plots in May 2015 in a native grass meadow at BEF. Dominant vegetation within the plots included Indian grass (*Sorghastrum nutans*), switchgrass (*Panicum virgatum*), big bluestem (*Andropogon gerardii*), musk thistle (*Carduus nutans*), wineberry (*Rubus phoenicolasius*), and milkweed (*Asclepias syriaca*). The plots were in fields with no other artificial lighting, and sky glow from human developments was low due to BEF's rural location.

The eight 20-m-diameter plots were grouped into four pairs, with a 10-m edge-to-edge distance between plots in a pair and a minimum of 20 m between pairs. Within

each pair, artificial light was added to one randomly chosen plot and the other plot, with no light added, served as a control. We erected wooden light posts in the center of each plot, including the control plots. At a height of 3 m, each light post was fitted with either four light-emitting diode (LED) waterproof floodlights (RAB Lighting Bullet 12A) or a fake light fixture. We placed posts fitted with fake light fixtures in the control plots to avoid differences in physical structure between treatments because we observed that potential predators of fireflies (birds and spiders) frequently perched on the light posts in pilot studies. We chose LED lights because they overlap firefly flashes spectrally (Elvidge et al. 2010; Lall and Worthy 2000), and because these technologies are becoming increasingly common in residential and commercial areas (Steele 2010). We chose a 10-m radius for the circular plots because this area could be effectively lit by the light fixtures, and was thought to be a relevant scale for firefly movement. A light sensor turned the floodlights on at dusk and off at dawn throughout the seasonal window of local adult firefly activity (mid-May up to and including early August). We quantified illuminance within each plot at several distances from the plot center with a light meter (ExTech LT300) at a height of 1 m above the ground on a cloudless night with a new moon. To capture variation in illuminance due to the angle of the light sensor, light measurements were taken with the sensor pointed upward and with the sensor pointed directly at the light. Using the latter method, illuminance was 301.24 ± 89.07 (1 SD) lux at the center post in plots with artificial light and 0.09 ± 0.10 lux in plots with no light (Fig. 1).

Adult firefly flashes were observed 1 night each week from 8 June to 24 July. After 24 July, *Photinus pyralis* flash activity at BEF was 13 % of peak and *Photuris versicolor* flash activity was 1.5 % of peak. We measured the number of firefly flashes per minute in each plot at sunset, 1 h after sunset, and 2 h after sunset based on the mean of three 1-min counts per time period. For each count, an observer stood at one of four cardinal directions around the plot periphery. Between each count, the observer either moved to a new, randomly selected location on the plot perimeter, or waited 30 s before beginning the next count in the event that the same location appeared consecutively in the random sequence. The purpose of moving randomly between counts was to ensure that the counts were not biased according to the location of the observer, for example due to obstructions presented by vegetation.

Visually surveying firefly flashes is a standard method for assessing local abundances (Takeda et al. 2006; Cratsley and Lewis 2005; Lewis and Wang 1991; Yuma and Ono 1985; Hori et al. 1978). We tested the assumption that differences in the rate of firefly flashing (e.g., flashes per minute) among locations reflect differences in abundance by

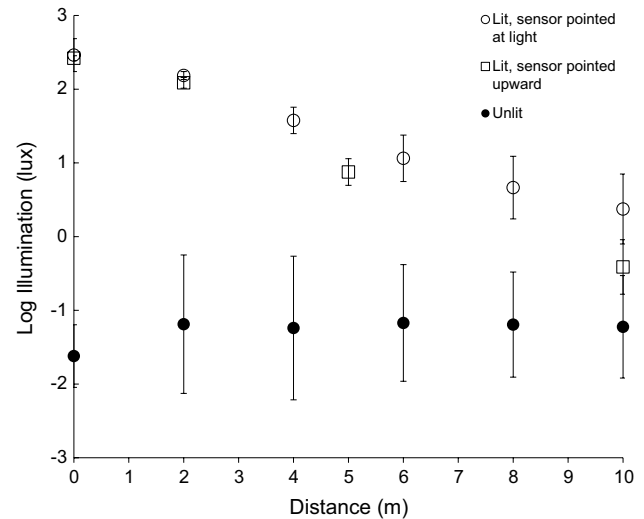


Fig. 1 Illumination (lux) in the experimental plots used for the firefly abundance experiment. Light levels were measured with the light sensor angled towards the artificial light and with the light sensor angled directly towards the sky. Data are means \pm 95 % confidence interval (CI)

comparing visual counts of flashes with a non-visual measure of firefly abundances. Two concerns motivated this test. First, differences in the rate of flashing among plots might be influenced by the propensity of individuals to flash, as well as firefly abundance. Second, it is plausible that firefly flashes may be easier for researchers to detect in darker locations due to the higher contrast between the flashes and the background. For our non-visual method, we completed sweep net samples of all of the non-*Rubus* vegetation (~20 % of the area in each of two paired plots) within each plot at waist level three times during the summer. Plots were sampled at sunset and 1 h after sunset. Fireflies were released back into the plots where they were captured at the end of sampling each night. We decided to use sweep net sampling instead of LED firefly traps (Bird and Parker 2014; Woods et al. 2007) as a non-visual abundance metric to avoid destructively sampling the population, and because our experimental manipulation of light levels could conceivably affect the attractiveness of the LED lure (Bird and Parker 2014).

As ambient light levels decreased over the course of each evening, it became increasingly difficult for researchers to see the plot boundaries. To ensure firefly flashes were counted within a standard area in both treatments, we marked plot boundaries with blue, chemiluminescent glow sticks before each survey. Fireflies may be insensitive to blue wavelengths of light (Lall and Worthy 2000), and the presence of glow sticks did not change light levels at the plot boundaries by more than 0.01 lux, the minimum illuminance detectable by our light meter.

To test the effects of light pollution on the flash activity of a given species (*Photinus pyralis* or *Photuris versicolor*) over the course of the summer, we used a generalized linear mixed model (GLMM) with light pollution, week, and light pollution \times week interaction as fixed effects, and plot pair as a random effect. The dependent variable was the mean of the number of firefly flashes per minute. We specified a Poisson distribution for the dependent variable and log-link function. To test whether effects of light pollution on firefly flash activities changed with time of night for a given species, we used a GLMM with light pollution, observation round (i.e., sunset, 1 h after sunset, 2 h after sunset), and light pollution \times observation round as fixed effects, and plot pair as a random effect. As before, we specified a Poisson distribution for the dependent variable with a log-link function. All statistical analyses were conducted using SAS software (SAS Institute, Cary, NC).

We also tested the assumption that firefly flash counts are a reasonable proxy of local abundances by evaluating the relationship between the mean number of *P. pyralis* flashes per minute over the course of the summer and the number of *P. pyralis* captured using a Pearson correlation. Both variables were log-transformed to meet the assumptions of the test. These data were pooled across all sample periods due to low overall numbers of captured individuals. Sweep net capture of the second firefly species, *P. versicolor*, was too low to analyze statistically.

Mating behaviors and mating success

To assess how light pollution affects *P. pyralis* mating behavior, we observed courtship dialogues between free-flying males and tethered female *P. pyralis* in the field with and without artificial light between sunset and 2 h after sunset (2030–2230 hours). This experiment was conducted in a native grass meadow that was continuous with the meadow containing the plots for the local abundances experiment, at a distance of 200 m from these plots. Female tethering locations were grouped in pairs with a distance of 20–30 m between points within a pair. We randomly assigned one point within a pair to receive light pollution. The light-pollution treatment consisted of two LED floodlights (Ultra-Tow 9-32 V LED Floodlight) mounted to a post at a height of 3 m with a mean illuminance of 167.21 ± 1.61 (1 SD) lux. Lights were powered with 12-V, 35-A batteries. Because researchers trampled some of the vegetation while setting up the light fixtures, we also walked around the control points several times to similarly disturb plots in both treatments.

We collected female *P. pyralis* for the experiment at BEF within 24 h of each trial. We tethered each female to a perch consisting of a 4×20 -cm wooden platform on top of a 0.5-m garden stake just prior to twilight and the onset of

firefly activity. Perches were positioned 2 m away from the light post or control point. A 30-cm length of cotton sewing thread was looped around a joint between the female's pronotum and abdomen to form a noose, with the leading end of the noose positioned dorsally. Females could walk normally while tethered.

A female was allowed to acclimate for 5 min before the observation period began. We then observed each female continuously for 15 min. The observer sat 2 m away from each tethered female and recorded several metrics of mating activity: the number of times the female flashed, the number of males flying within 1 m of the female, and the number of times males were within 1 m of the female and flashed. We carried out 27 replicate mating trials for each treatment, observing a unique female in every trial.

Differences in mating behavior metrics were assessed with a multivariate ANOVA (MANOVA), with light treatment as a fixed effect and pair as a random effect. All of the response variables were log-transformed to improve normality and homogeneity of variance. Following the finding of a significant multivariate response, univariate ANOVAs were also performed using each of the response variables (Quinn and Keough 2002).

Dispersal

To study whether fireflies tend to move towards or away from artificial light, we released marked male *P. pyralis* fireflies in plots with light pollution added and control plots with no added light and monitored firefly displacement from the release point. Again, we used *Photinus pyralis* instead of *Photuris versicolor* in this experiment for logistical reasons. *Photinus pyralis* was more abundant than *Photuris versicolor* at BEF, and we were unable to capture *P. versicolor* in sufficient numbers for a mark–release–recapture experiment. Paired release sites were established on lawns at BEF, with 80 m between paired release sites and at least 300 m between pairs of release sites. Light-pollution treatment levels (light polluted or control) were randomly assigned to the points within each pair. Light pollution was added with two LED floodlights mounted on a post at a height of 3 m, with a mean illuminance of 167.21 ± 1.61 (1 SD) lux. LED lights were turned on at dusk to dawn during the experiment. A barrier (i.e., road, hill crest, clump of trees) always separated the light-pollution treatment and control release points so that they were never within sight of each other.

Male *P. pyralis* fireflies were captured at night 24 h prior to the release, marked with fluorescent powder, and housed in outdoor containers. At sunset the following evening, we released 100 males at each point within a pair, using a different color of marking for each point. In a single night, releases were carried out on one to two plot pairs.

Twenty-four hours after a release, all live *P. pyralis* fireflies were recaptured within two distance ranges from each release point: 0–8 and 8–16 m. Pilot studies showed that attempting to recapture at farther distances was too time consuming to complete within the short nightly activity period of *P. pyralis*. The time spent collecting within each distance section (7 min 30 s in the inner section, 22 min 30 s in the outer section) was adjusted based on the area of that section to standardize the sampling effort. Paired plots were sampled by two people simultaneously (one collector per plot). The collectors switched plots halfway through the sampling period to correct for potential differences in sampling efficiency between individuals.

The collectors typically caught all of the fireflies that were visible in a particular distance range before the sampling time period ended. For this reason, we judged that standardizing the number of recaptures by the area searched (201.6 or 603.19 m²) was the least biased estimator of recaptures. To test for effects of light pollution on firefly displacement from the release point, we used a GLMM with light pollution, recapture distance, and the light pollution × recapture distance interaction as fixed effects and pair and plot as random effects. The response variable was the number of marked fireflies recaptured per squared meter. This was square-root transformed to meet normality and homogeneity of variance assumptions. The hypothesis that light pollution affected displacement distance was tested based on the light pollution × recapture distance interaction. In the process of recapturing marked fireflies, collectors also captured unmarked fireflies. We tested effects of light pollution on the distribution of the unmarked fireflies (males captured^{0.5}/m²) with a second GLMM with the same fixed and random effects as the previous model.

Results

Flashing activities and abundances

We counted a total of 10,699 and 903 *Photinus pyralis* and *Photuris versicolor* flashes, respectively, over the course of the 7 weeks of this experiment. The median number of flashes observed per minute was 22.29 for *Photinus pyralis* and 1.88 for *Photuris versicolor*.

Light treatment did not significantly explain differences in the mean number of *P. pyralis* flashes per minute over the 7 weeks of the experiment (Fig. 2). For this species, flash activity peaked in week 4 of the study, and week explained a significant portion of the variation in flash counts (Table 1). Within nights, the greatest number of *P. pyralis* flashes was observed at sunset, but light pollution

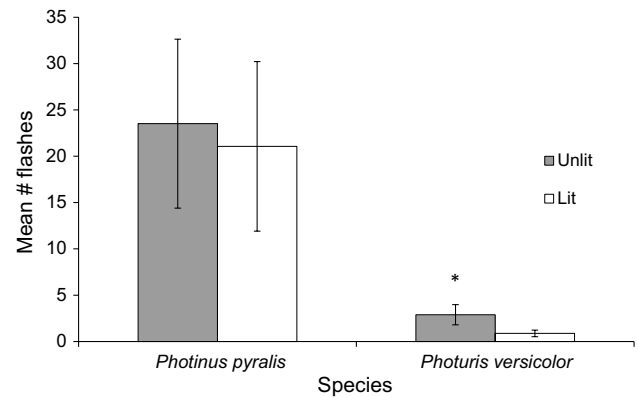


Fig. 2 The effect of light pollution on the number (hash symbol) of *Photinus pyralis* and *Photuris versicolor* flashes per minute in the flashing activity and abundance experiment [generalized linear mixed model (GLMM), statistical results in Table 1]. The nightly mean number of flashes per minute was calculated for each plot by taking the average of three 1-min observations made at sunset, 1 h after sunset, and 2 h after sunset. Nightly means were then averaged across the 7 weeks of the study. Data are means ± 95 % CI. Asterisk denotes a statistically significant finding at the $\alpha = 0.05$ confidence level

Table 1 Results of generalized mixed models testing the effects of light pollution on firefly flashing activity and abundance

Species	Variable	F_{df}	p
<i>Photinus pyralis</i>	Week	$F_{6,42}$	3.24 0.011*
	Light pollution	$F_{1,6}$	1.75 0.23
	Week × light pollution	$F_{6,36}$	0.16 0.99
	Observation round	$F_{2,6}$	55.38 ≤0.01**
	Observation round × light pollution	$F_{2,6}$	0.54 0.61
<i>Photuris versicolor</i>	Week	$F_{6,36}$	2.37 0.05*
	Light pollution	$F_{1,6}$	10.55 0.02*
	Week × light pollution	$F_{6,36}$	0.31 0.93
	Observation round	$F_{2,6}$	2.04 0.21

* Significant at the $\alpha = 0.05$ confidence level, ** significant at the $\alpha = 0.01$ confidence level

neither delayed nor advanced the timing of *P. pyralis* activity (Table 1).

Light pollution strongly affected mean *P. versicolor* flashes per minute, reducing the number of *P. versicolor* flashes observed in the control plots by 69.69 % compared to the light-pollution plots (Fig. 2). *P. versicolor* flash counts peaked in week 1 of the study. Week explained a significant portion of the variation in flash counts, but there was not a significant week × light pollution interaction (Table 1). Within nights, there was not a significant effect of observation round (i.e., sunset, 1 h after sunset, 2 h after sunset) on *P. versicolor* flash counts (Table 1).

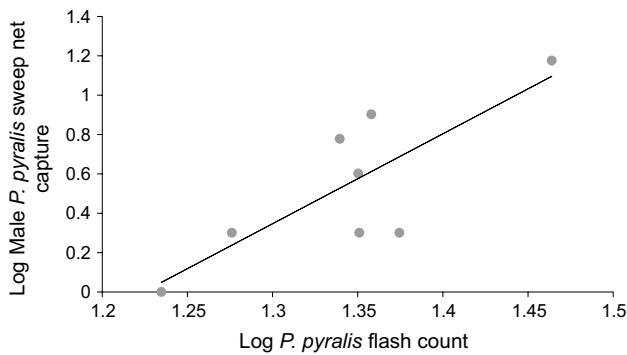


Fig. 3 Scatterplot and trend line of the relationship between the mean number of flashes observed in each plot over the course of the summer and the number of male *P. pyralis* captured in sweep-net surveys (both log-transformed; $r = 0.78$, $n = 8$, $P = 0.020$)

The mean number of *P. pyralis* flashes observed per minute in each plot over the course of the summer was positively correlated with the total number of *P. pyralis* ($r = 0.77$, $n = 8$, $P = 0.025$) and the number of male *P. pyralis* (Fig. 3, $r = 0.78$, $n = 8$, $P = 0.020$) captured in sweep-net surveys (all variables log-transformed). As we found with the flash count surveys, there was no significant difference in total number of male *P. pyralis* captured between light-pollution treatment levels (paired *t*-test, $P = 0.72$, $t = 0.39$, $df = 3$).

Mating behaviors and mating success

On average, 4.10 male *P. pyralis* flew within 1 m of each tethered female during the 15-min observation periods. Of these, 94 % males flashed at least once. The median number of flashes observed per male was 3. Forty-three percent of tethered females flashed at least once. Among females who flashed, the median number of flashes was 2.

Based on a MANOVA, we found a multivariate effect of light pollution on *P. pyralis* mating behavior (Wilks' $\lambda = 0.814$, $F_{4,51} = 2.89$, $P = 0.031$). Univariate ANOVAs

of each of the response variables revealed that females were three times more likely to flash in the control treatment (Fig. 4a; $F_{1,54} = 6.84$, $P = 0.012$). There was no significant difference in the number of males flying within 1 m of the female (Fig. 4a; $F_{1,54} = 1.30$, $P = 0.26$), or the proportion of those males that flashed (Fig. 4b; $F_{1,54} = 2.10$, $P = 0.15$).

Five females mated with males during the observation periods and reached the stage of copulation in which spermatophore transfer occurs (Lewis and Wang 1991). All females that mated were in the control treatment.

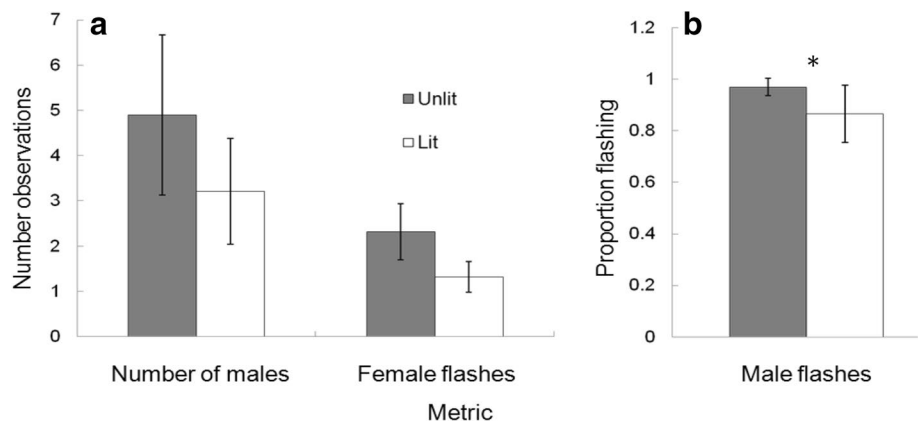
Dispersal

We recaptured 237 out of 2,000 marked *P. pyralis*, or roughly 12 % of those released. Recapture rates ranged from 1 to 44 %, with a mean of $11.92 \% \pm 9.75$ (1 SD). There was no significant effect of light-pollution treatment on displacement distance (light pollution \times distance; Fig. 5a; $F_{1,18} = 0.16$, $P = 0.69$). We also captured 1613 unmarked *P. pyralis* while sampling during the course of the experiment. There was no significant effect of light pollution on the mean number of unmarked fireflies captured ($F_{1,18} = 0.29$, $P = 0.60$; Fig. 5b), nor was the light pollution \times distance interaction significant ($F_{1,18} = 0.17$, $P = 0.21$).

Discussion

To date, research on the ecological consequences of light pollution has focused mainly on organism-level effects such as changes in physiology or behavior, with little focus on the implications for populations (Gaston and Bennie 2014). Few studies have demonstrated impacts on species abundances or distributions with controlled experiments. One notable exception is that of van Geffen et al. (2015), which showed that LED lights reduced mating success in a geometrid moth. Here we report on some of the first

Fig. 4 Effect of light pollution on **a** male *P. pyralis* abundances (ANOVA, $F_{1,54} = 1.30$, $P = 0.26$) around tethered females and number of female response flashes (ANOVA, $F_{1,54} = 6.84$, $P = 0.012$) and **b** proportion of male *P. pyralis* that flashed within 1 m of a tethered female (ANOVA, $F_{1,54} = 2.10$, $P = 0.15$). Data are means \pm 95 % CI. Asterisk denotes a statistically significant finding at the $\alpha = 0.05$ confidence level



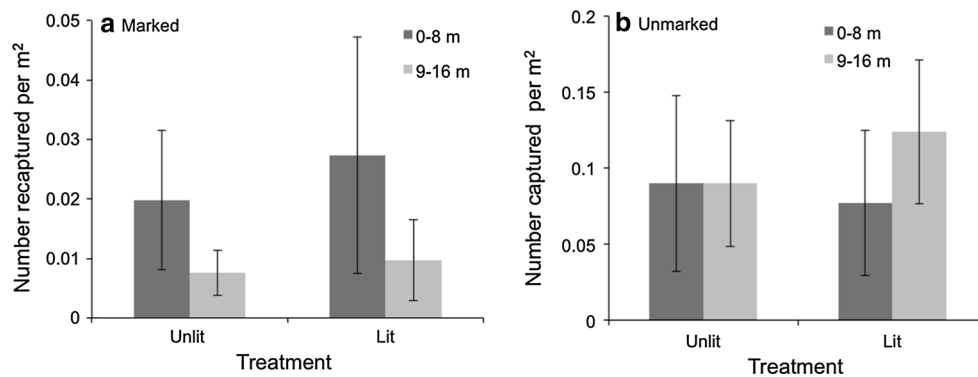


Fig. 5 a Effect of light pollution on male *P. pyralis* dispersal (GLMM, $F_{1,18} = 0.16$, $P = 0.69$). Fireflies were either released directly under a light source (light-pollution treatment) or at a point with no light source (control). Darker grey bars represent the num-

ber recaptured per minute 0–8 m from the release point and lighter grey bars represent the number recaptured 9–16 m from the release point. **b** Effect of light pollution on captures of unmarked *P. pyralis* (GLMM, $F_{1,18} = 0.17$, $P = 0.21$). Data are means \pm 95 % CI

experimental testing of effects of light pollution on local firefly abundance, as well as mechanisms (dispersal and mating success) potentially influencing abundance.

We demonstrated that light pollution disrupts female firefly courtship flashes (Fig. 4a) and mating success (mating only occurred in control plots), which may in turn lead to reduced rates of reproduction and population growth in light-polluted areas. The reduction in mating success appeared to be driven by changes in the behavior of females rather than males. Male *P. pyralis* were no less active or abundant in light-pollution plots, and our dispersal experiment results produced no evidence of males being attracted to, or repelled by, artificial light. While the continued courtship activity of *P. pyralis* in light-pollution conditions appears counterproductive given that no mating occurred, the fact that males were not attracted to the artificial lighting may indicate that the impacts of light pollution on *P. pyralis* populations to light-polluted areas are limited. The lack of attraction to artificial lighting in fireflies is in contrast with the attraction of other species to artificial light [e.g., some moths, beetles, and aquatic insects (Eisenbeis 2006; Perkin et al. 2014)], where aggregation in light-polluted areas could create population sinks. Studies that document spatial (immigration and emigration) as well as non-spatial components (births and deaths) of demography will be most informative for inferring population-level impacts of light pollution.

Perhaps uniquely, we use two methods to assess firefly abundances: visual counts of firefly flashes and sweep-net capture. We found that sweep-net capture was positively correlated with flashes per minute, suggesting visual surveys of firefly flashes are a reasonable proxy for *P. pyralis* abundances. When possible, we recommend non-visual surveys should be used to complement visual surveys when measuring firefly abundances. The combination of flash counts and sweep-net surveys allows us to conclude the

local abundance of *P. pyralis* was not affected by light pollution over the span of a single adult generation. However, it is important to consider that effects on abundance might arise over a longer time frame if the observed reduction in mating success inhibits reproduction.

Our finding that light pollution did not affect male *P. pyralis* abundances near tethered females contrasts with Bird and Parker's (2014) observation that males of *Lampyrus noctiluca*, a European firefly species, were less attracted to simulated females in the presence of light pollution. Previous laboratory work with *L. noctiluca* also shows that artificial light decreases female bioluminescent activity (Dreisig 1975), a finding echoed in our study with *P. pyralis* females. Despite the different responses of *L. noctiluca* and *P. pyralis* males, and although female bioluminescence behaviors differ for these two species—*L. noctiluca* females produce a continuous glow while *P. pyralis* females produce discrete flashes—light pollution may have a similarly negative impact on mating success in these two species.

We expect that a reduction in courtship dialogues may have complex effects on individual fitness in *P. pyralis*. In many firefly species (including *P. pyralis*), females mate multiple times and gain supplemental resources with each additional mating in the form of a male nuptial gift (van der Reijden et al. 1997; Lewis et al. 2004). The fitness pay-off of mating multiple times, and penalty of failing to do so, may be particularly large in this system because *P. pyralis* do not eat as adults. Our findings suggest future avenues of research exploring the effects of light pollution on *P. pyralis* fecundity, and in turn, on the growth and dynamics of firefly populations in affected areas.

In contrast to *Photinus pyralis*, light pollution resulted in decreased flashing activity in *Photuris versicolor*. *Photinus pyralis* is generally active earlier in the evening than *Photuris versicolor* when natural light levels are higher, but are

rapidly decreasing. Divergent responses to light pollution among twilight- and dark-active fireflies may reflect differences in the ability of these species to tolerate diverse light conditions, or the fact that ambient light levels are simply higher earlier in the evening and may already exceed light levels from artificial light illumination. In other systems, light pollution has also been found to affect nocturnal species more strongly than crepuscular species (Azam et al. 2015; Rotics et al. 2011). Temporal niche may be an important trait influencing how a species will respond to light pollution.

Our observation that light pollution reduces firefly mating opportunities joins a chorus of other examples of communication systems disrupted by sensory pollution. Acoustic pollution has been shown to alter signal characteristics and behavior in birds (Slabbekoorn 2013; Proppe et al. 2013), crickets (Costello and Symes 2014), whales (Miller et al. 2000), and frogs (Bee and Swanson 2007). Nutrient pollution reduces the efficacy of a colored sexual display in sticklebacks (Wong et al. 2007; Candolin et al. 2007). Atmospheric pollutants can degrade plant volatiles (McFrederick et al. 2008; Girling et al. 2013), with the potential for cascading effects on the networks of pollinators and herbivores that depend on them. Because communication often plays a role in mate-finding and assessment, there is a clear need to explore the implications of sensory pollution for individual fitness and demographic processes separate from other stressors than may occur in human-modified landscapes.

We show that anthropogenic disturbance in the form of light pollution reduces flash activities in a dark-active firefly species and mating success in a twilight-active species. These results suggest that light pollution has the potential to adversely affect firefly populations, and point to the need for additional work on the effects of increasing urbanization of landscapes on mating behavior and the fallout for population persistence. In the case of *P. pyralis* populations, adverse effects of light pollution would not have been noticed if our inferences had been based solely on abundances, and reductions in mating success had not been detected.

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Author contribution statement A. F. and K. H. conceived and designed the experiments. A. F. performed the experiments. A. F. and K. H. analyzed the data and wrote the manuscript.

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