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SHORT COMMUNICATION

Low levels of light pollution may block the ability of male glow-worms (*Lampyris noctiluca* L.) to locate females

Stephanie Bird · Joel Parker

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Abstract Light pollution has been proposed as a factor in the decline of *Lampyris noctiluca* because it has the potential to interfere with reproductive signaling and has been shown to impact the ability of males to locate light lures in a suburban environment. To compare and test the replicability of this effect in a natural setting and population, imitation females were set out under light polluted and control conditions at varying light pollution intensities in an undisturbed British chalk grassland. Very low levels of light pollution were found to interfere with phototaxis: no males were attracted at either 0.3 or 0.18 lux background lighting versus 33 males collected at paired dark controls. These background illumination levels are much lower than that of 1.5 lux which is recommended by local city councils in Britain to light footpaths. A survey of female *L. noctiluca* numbers and distribution showed a trend towards female clumping that was not statistically significant. We also found no evidence of light interfering with female signaling behavior.

Keywords *Lampyris noctiluca* · Glow-worm · Light pollution · Phototaxis · Distribution

S. Bird · J. Parker
Centre for Biological Sciences, University of Southampton, Southampton SO17 1BJ, UK
e-mail: stephanie.bird@roehampton.ac.uk

Present Address:
S. Bird
University of Roehampton, London SW15 4JD, UK

Present Address:
J. Parker (✉)
Department of Biological Sciences, State University of New York, Plattsburgh, 101 Broad Street, Plattsburgh, NY 12901, USA
e-mail: joel.parker@plattsburgh.edu
URL: http://www.jparcoeur.com

Introduction

Habitats such as the unimproved chalk grasslands of Great Britain are under threat from grazing mismanagement, agricultural intensification and industrial/urban development. Most of the human impacts studied concern the effects of agricultural practices such as stocking and nitrification from fertilizers which alter the local bird, vegetation and insect population compositions (Vickery et al. 2001; Critchley et al. 2004). One less examined aspect of human impact is the additional light pollution surrounding these areas as they are often close to towns or lit roads and pathways.

There is a growing awareness of ecological light pollution impacts on insect populations. Lights can attract insects, moths in particular, concentrating them for predation (Rydell 1992) or death through exhaustion as when attracted to street lights (Eisenbeis 2006). Another example is the reflection of polarized light from the surface of asphalt mimicking water, drawing in aquatic insects for predation (Yoon et al. 2010). Artificial lighting can also disrupt animal foraging, migration and reproductive behavior (Longcore and Rich 2004; Stone et al. 2009; Ineichen and Rüttimann 2012). Many of these negative effects are dependent upon specific wavelengths of light and not necessarily intensity (Longcore and Rich 2004). Thus altering light wavelengths could be an option where safety concerns may not allow light removal. Recent work suggests that the physiology of different types of photoreceptors in an insect's eye might contribute to wavelength specific effects of light pollution. For example, the eyes of the European glow-worm, *Lampyris noctiluca L.*, have distinct green and blue photoreceptors (Booth et al.

2004). In this case, excitation of the blue receptors can interfere with the male preference associated with the green receptors. Excess of particular wavelengths of light in addition to intensity may interfere or block natural responses to the bioluminescence that *L. noctiluca* use in their mating behavior.

Lampyris noctiluca is one of only two Lampyridae species found in the United Kingdom. There is a great deal of interest in these charismatic animals in Great Britain that has led to an online citizen science project, the UK glow-worm survey (http://www.glowworms.org.uk/), for long term monitoring of glow-worm populations. Populations in Great Britain are declining (Tyler 1986, 1994, 2004; Alexander 1992; Gardiner 2007a, b) and it has been proposed that light pollution may be a contributing factor (Tyler 1986) due to *L. noctiluca*'s use of bioluminescence to find mates.

Female *L. noctiluca* produce a continuous cold light via a series of chemical reactions to attract males in contrast to fireflies that can modulate the light emitted resulting in species-specific flash patterns (Branham and Wenzel 2003). This bioluminescence only occurs at night, when the females are active, as they remain under/amongst cover during daylight to avoid predation (Gardiner 2007c). Adult glow-worms appear to have evolved many predation avoidance mechanisms and even some that may be enhanced by glowing (Day 2011 for review). Females appear and may glow each night of their adult phase until mated, which lasts from late May until early August, depending on latitude (Tyler 1986, 2002; Sala-Newby et al. 1996; Gardiner 2007b). During this period, the females climb up to the top of a grass stalk or other suitable prominent structure to become more visible and then twist to expose their underside, orientating their luminous organs upwards (Schwalb 1961). This bioluminescent process starts after the light levels have decreased below a critical threshold, typically during or just after twilight. However this process is also reliant upon a natural circadian rhythm of sensitization, during which the female becomes more receptive during ambient light levels (Driesig 1975). More recently it was suggested that a trade-off between achieving maximum body size and timing of mating plays a role in the seasonal timing of female glowing (Horne 2011) with females aligning their appearance with males that appear later in the season and disappear earlier (Horne 2011; Tyler 2011).

Early field work on the question of light interference with glow-worm mating behavior has focused on the intensity of lighting. It has been shown that males react positively to light intensities up to about 200 lux, but at higher intensities they either do not respond, or react negatively (Schwalb 1961). Both male and female glow-worms could be attracted to the wrong habitat by artificial light. The males would perceive it as a colony of brightly glowing females, while females would assume it was an appropriate site for oviposition (Lloyd 2006). There are also examples of the effects of light pollution on females; Schwalb (1961) established that the sexual appetency behavior of the females was disturbed at light intensities over 80 lux and that overhead lighting of 500 lux causes females to retreat and to cease glowing. In contrast, Lloyd (2006) found light attractive to females. Driesig (1975) reported that some females did not commence their reproductive behavior with ambient light over 1 lux, and observed no activity above 10 lux. This falls in the mid-range of recommended lighting levels for subsidiary roads and pedestrian areas (The Institute of Lighting Engineers (Great Britain) 2005). In a suburban area, light levels of 46–64 lux were shown to completely block males from finding light traps directly under street lighting with the effect disappearing at 04–0.1 lux between the lights (Ineichen and Rüttimann 2012).

Even if the males are not attracted to the light pollution source, such a source could still interrupt reproductive behavior. Longcore and Rich (2004) suggested that stray light could wash out the visual signals between males and females leaving both unable to perceive the less intense natural light of the females against that of more powerful streetlamps. This hypothetical effect would likely be involved in the earlier mentioned effect where blue wavelengths (485 nM) interfere with the signals of the target green wavelengths (555 nM) in *L. noctiluca* (Booth et al. 2004).

This study had three objectives: the first was to assay the size and distribution of the female glow-worm population over the breeding season at Brush Hill, the second to test if light pollution might interfere with signaling females avoiding each other and finally to test whether an artificial light source can interfere with male phototaxis while they are searching for females.

Methods

Study site

The study was carried out at the Brush Hill Nature Reserve, SP 821 034 GB, an unimproved chalk grassland site on Brush Hill, near Princes Risborough, in Buckinghamshire (59° 43′26.89N, 0° 48′45.69W). The study was conducted from June 22nd to August 2nd, 2007. Weather and cloud cover data was collected over the study period (data not presented).

General female survey

To aid in the UK glow-worm survey, glowing females were counted along established transects each night beginning

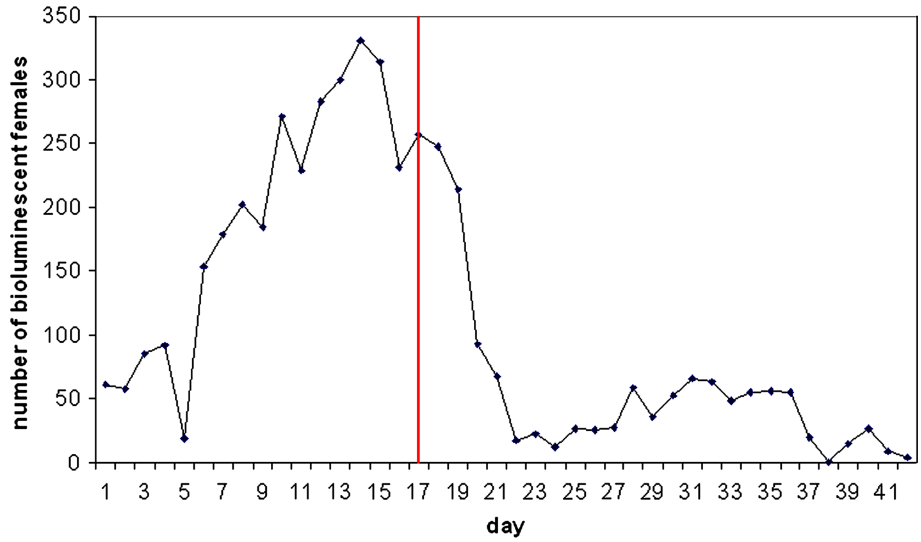

Fig. 1 Numbers of bioluminescent females found in the sample area over time, *vertical line* indicates first appearance of males

on June 22nd, 2007 nightly for 42 days across a set area of the hill-side one to 2 h after sunset when the females started glowing (Fig. 1). The date when males first appeared was noted. During one night, the map locations were recorded and a grid was superimposed to determine the dispersion of females.

Effect of light on females

To test whether females might be inhibited by nearby glowing females, imitation females were constructed using green 5 mm light emitting diodes (LEDs) (WL28, peak 565 nM) to match previously measured wavelengths emitted by natural females (550–570 nm) (Schwalb 1961; Sala-Newby et al. 1996; Booth et al. 2004; Tyler 2011; Ineichen and Rüttimann 2012). These were soldered to a 2 cm by 1.5 cm circuit board, fitted with a mini resistor (make; M270R) and attached to a 9 V battery to create a circuit. Green garden canes were cut to a height of 30 cm to simulate grass stalks. The LEDs were then attached to the top and bound on with duct tape. The canes were planted so that the LEDs were approximately 9 cm above the ground based on observations of the height females were climbing in the area. One of these artificial lights on a cane was placed beside 12 different females and the length of time the females continued glowing was measured. The mean time to cessation of bioluminescence was calculated.

Measuring effect of light pollution on males

A transparent plastic cup was then attached to the artificial females described above at the top of the garden cane so it surrounded the LED, creating a pitfall-like trap, with small holes for drainage in case of rain. A 'white light' pollution source consisting of a standard 9 volt filament bulb torch was used as it most closely resembles the mercury or ceramic metal halide (CMH) lights that are currently used to replace the older low pressure sodium bulbs (Adams, M. Lighting Manager, Southampton City Council, pers. comm.). Using the same light meter employed by the local council to assess street and path lighting (Hagen Minilux 2 light meter, GEC Instruments), imitation females were set up at the following distances from the light pollution source at lux levels: 0.5 m (0.3 lux), 1 m (0.18 lux), 1.5 m (0.09 lux), 2 m (0.07 lux) where lux is measuring the light emitted by the light pollution source at the location of the artificial female. This range was the highest obtainable and chosen to fall within the levels of ambient starlight at 0.0009 lux and footpaths at 1.5 lux, (Adams pers. comm.) This set up was duplicated at a similar location on the site. The experiments were started between 10:45 pm and 11:15 pm each night and males drawn to the artificial females were counted after 2 h. Males were held until the end of each night and released to avoid resampling. Abiotic factors including weather and moon phase were recorded. A paired design was employed with a light polluted and non-polluted trial run simultaneously each night, switching locations between experimental and control on subsequent days.

Statistical analysis

To test whether the observed distribution of females was clumped, random or overdispersed in the mapping study, the coefficient of dispersion (CD) was calculated and a two tailed Chi square was performed (Grafen and Hailes 2003).

The mean time females persisted in glowing in the presence and absence of the artificial lights was tested with a Student *T* test.

In order to test the effects of light pollution on males, the data were tested for normality in Minitab, with nonparametric

Table 1 Light pollution interferes with male location of artificial females

Lux	N	Number of males at light polluted positions	Number of males at non-polluted positions	Median lit	Median unlit	*P* value
0.3	12	0	33	0	2	<0.0025*
0.18	12	0	33	0	3	<0.0025*
0.09	12	6	26	0	2	<0.001**
0.07	12	4	6	0	0	n.s.**

* Wilcoxon test against a mean of zero

** Mann–Whitney test

Wilcoxon and Mann–Whitney tests being conducted. Ideally a two-way analysis of variance would have been performed, with the light pollution and distance from it as factors, however, the data was not normally distributed ($P < 0.005$). Therefore non-parametric tests were performed.

To better understand how interference falls away with light intensity in the male attraction experiment, the percentage inhibition relative to controls for the male glow-worms caught at each lux level were plotted. Matlab was used to fit several models to these data, before a custom model with 95 % confidence bounds was generated.

Results

General survey

A count of the females over the study period is shown (Fig. 1). The numbers of females producing bioluminescence showed an upwards trend until day fourteen, to a maximum of 331 individuals, with the only major departure coming on day five. At this point, numbers declined for the next 2 days before rising again on day seventeen. Day seventeen was also the day when the presence of males was first noted, more than 2 weeks later than the females. Female numbers then decreased, although more do appear towards the end of the glowing season, they fail to reach a third of the number seen at the peak (day fourteen).

The survey results were mapped over the study site. The distribution of displaying females was not significantly clumped although there is a trend in that direction (Chi squared $= 53.6923$, d.f. $= 37$, $P = 0.075$) with a CD of 1.429.

Effect of light on females

The LED lighting on the canes had no effect on the duration of female bioluminescence, as demonstrated with a student *T* test ($T_{22} = 1.66$, $P = 0.112$, two-tailed). There was however a trend in the direction of females ceasing to glow in the presence of artificial females (LED canes: mean of 28.6 min, standard deviation of 15.8 and control canes: mean of 46.8 min, standard deviation of 34.6 min).

Measuring effect of light pollution on males

Very low levels of light pollution significantly interfered with males finding the artificial females (Table 1). Out of a total 108 male *L. noctiluca* attracted to imitation females at varying distances from the light pollution source or centre of the circle, only 10 males were found on the LEDs under any light polluted conditions. No males were attracted to the imitation females at 0.3 or 0.18 lux versus 33 males attracted to each of the two paired controls. A custom model with 95 % confidence bound was generated was generated for these data to better visualize the effect of light pollution on males [$f(x) = a*\exp(-b*x) + 100$, depicted in Fig. 2]. The figure (including the four data points) shows the correlation between light level and how males are not finding the traps with 100 % interference from 0.2 lux and above.

Discussion

Tyler (1994) noted that during a 1992 glow-worm survey, most of the sites were situated 'well away from any form of artificial lighting'. A more recent survey of *L. noctiluca* in Essex showed that the majority of sites (78 %) were found in areas of no artificial lighting, including nature reserves (Gardiner et al. 2002), indicating that lack of light pollution may be an important constituent for a suitable *L. noctiluca* habitat. The fact that glow-worms tend to occur away from artificial lights has a more positive interpretation, as area use will largely determine artificial lighting. Areas where artificial lights are needed for human activity are less likely to consist of natural grassland habitat suitable for glow-worms. On the other hand, if developments are being considered in or around areas where glow-worms are known to occur then understanding how artificial lighting might impact the populations should be a critical factor in deciding the level and type of lighting used.

Despite poor, wet, weather during the summer of 2007 we found a maximum of 331 signaling females on one night at Brush Hill indicating that it supports a glow-worm population of considerable size (Fig. 1). The number of female glow-worms found at established sites can vary

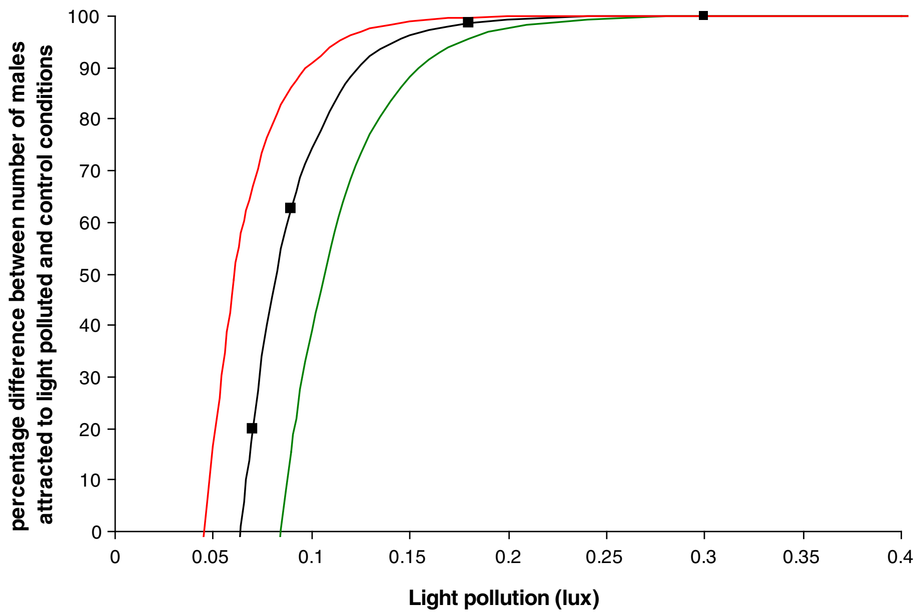

Fig. 2 A rapid increase in interference by artificial light blocking the ability of males to find the light traps. The points are data and the *lines* represent the curve fit and 95 % confidence intervals around the fitted curve

hugely from year to year (Tyler 1986; Hickmott and Tyler 2011). Figure 1 shows how the number of females varied in our study over the 42 day survey period with a drop off occurring around day 19 when the first males appeared as expected because mated females cease to glow. Weather is another factor that has been shown to effect glow-worm activity and it appeared to play a role in this survey as well. The high number of females observed on day 13 may be partially attributed to the poor weather conditions preventing males from flying and leaving unfertilized females glowing. This is supported by Gardiner (2006) who also found that female counts at a site in Essex were higher on nights with rain or drizzle, than without. The low numbers of females recorded on day five (nineteen individuals) could be explained by the weather data collected; the temperature only reached 9 °C and it was also remarkably bright due to no cloud cover and the approaching full moon. There was also a decrease in the number of females counted 2 days before the males were first observed (day seventeen). These results concur with Tyler (1986, 2011) where the males appeared after the females, although in our case they were observed 2 weeks later, a delay that could be the result of the adverse weather conditions experienced. After the males presence was first noted, the numbers of bioluminescent females decreased, as females were being fertilized, negating the need to glow. This means that the observation of many glowing females might mean less mated females rather than a healthy population.

No evidence of females' avoidance while signaling

Calculation of the CD on the GPS data did indicate clustering of the females which was not significant when a Chi square test was performed. If this trend was not due to chance then there are several possible reasons for the observed patterns. The female distribution could be a result of larval feeding locations or clumping to increase the chances of attracting males (easier to see a group from a distance than a single individual). There is no suggestion of the spreading out of females to avoid interfering with each other's signaling. We also found no significant effect of artificial female lights on female glowing time. Avoidance of predators due to light emission does not seem to be an issue because glow-worms have evolved many predatory avoidance methods (Day 2011). Taken together, we found no significant evidence at Brush Hill for females to be in competition with one another for signaling sites or effort to attract males.

Effect of light pollution on males

Even at very low levels, light pollution significantly affects the ability of male *L. noctiluca* to find females (Table 1), with the Matlab model providing an estimate for the difference in number of males attracted for a given level of light pollution (Fig. 2). This level of about 0.1 lux is fourfold lower than any light level previously observed to interfere with glow-worms. These levels corresponded to the light levels between lamp posts reported to have no discernable effect on male trapping rates in a suburban environment except for the background variation in males searching for mates (Ineichen and Rüttimann 2012). The intensity of light pollution used in our study was less than that normally employed to light the average footpath, yet it still interfered with the males' ability to find the imitation females. One important difference between the suburban study and our study is the orientation of the light. Our source was closer to the traps and directed upwards where as in the urban study, the street lamps were pointed downward with the traps set in the light cones. Earlier physiological work identified two types of photoreceptors in the male glow-worm interacting with each other (Booth et al. 2004). Thus it is equally likely the wave lengths as well as the intensity of light are acting to confound the males. The orientation difference in the two studies could be reducing male visits by dazzling (distraction or repulsion by a ground light source) or by a saturation effect (not discerning the light trap due to high background light). The two studies taken together suggest that ground based foot path lighting and overhead lighting will both prevent males from finding glowing females but possibly not by a common mechanism that can be assessed with a simple light intensity measure.

Our study utilized extremely simple methods and a basic light source (a standard 9 volt torch) yet we found significant results in the field. This work confirms that light pollution can interfere with the phototaxis of male *L. noctiluca* searching for female mates. If the number and size of *L. noctiluca* populations are declining, then light pollution in areas surrounding their habitats should be examined. Further studies need to be done to see how different types, intensities and light placements affect glow-worm mating both in the lab and in the field. If artificial lights are impacting glow-worm reproduction then knowing the specific mechanisms will be to addressing this form of pollution if these charismatic insects are to be preserved. Although the glow-worm is not a keystone species, no-one can deny that the British countryside would be a darker place without it.

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