White LED light intensity, but not colour temperature, interferes with mate-finding by glow-worm (Lampyris noctiluca L.) males

Reference:
Van den Broeck Mira, de Cock Raphaël, Van Dongen Stefan, Matthysen Erik.- White LED light intensity, but not colour temperature, interferes with mate-finding by glow-worm (Lampyris noctiluca L.) males
Journal of insect conservation - ISSN 1366-638X - 25:2(2021), p. 339-347
Full text (Publisher's DOI): https://doi.org/10.1007/S10841-021-00304-Z
To cite this reference: https://hdl.handle.net/10067/1774030151162165141

White LED light intensity, but not colour temperature, interferes with mate-finding by glow-worm (*Lampyris noctiluca* L.) males

Mira Van den Broeck[1*], Raphaël De Cock[1], Stefan Van Dongen[1], Erik Matthysen[1]

[1] Evolutionary Ecology Group, University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, B-2610 Wilrijk, Belgium

* Corresponding author: Mira Van den Broeck.
E-mail: miravdb@hotmail.com

Declarations

Funding : Not applicable

Conflicts of interest/Competing interests : The authors declare that they have no conflict of interest.

Ethics approval : No ethical approval was required for this study. No animals were harmed or killed, and all were released after capture

Consent to participate : Not applicable

Consent for publication : Not applicable

Availability of data and material : see Excel file "Data Van den Broeck et al. xlsx"

Code availability : Not applicable

Authors' contributions : E.M. and R.D.C. designed the study with input from M.V.d.B., M.V.d.B. prepared the field protocol and collected the data, M.V.d.B. and S.V.D analysed the data, M.V.d.B. wrote the article and all authors assisted during writing.

Acknowledgements

We would like to thank the numerous volunteers from Natuurpunt Londerzeel, family and friends who were involved in the fieldwork. With special thanks to Hilde Vervaecke for daily assistance during the fieldwork and preparations, and to Adriaan Peetermans for technical support.

Abstract

Artificial light at night is an increasing threat to nocturnal biodiversity. Aside from the overall increase in light emission, replacement of old monochromatic streetlighting by broad emission spectrum LED lights may be an additional threat. Studies evaluating the impacts of these artificial lights on the nocturnal European common glow-worm (*Lampyris noctiluca* L.) are scarce. This study examines the effects of upward facing white LED lights on the mate seeking activity of male glow-worms. Therefore we used traps with dummy females along a distance gradient from LED lights with different intensities and colour temperature (cold and warm white) and counted the number of males attracted per trap. We found that upward facing white LED light negatively impacted the males' ability to locate the females, at previously unreported low light levels, while colour temperature did not affect the outcomes. More research on the effects of light pollution and their underlying mechanisms is needed to evaluate the impacts of this emerging and widespread threat on mating success and population persistence of glow-worms.

Implications for insect conservation: Our study has important implications for glow-worm conservation as we showed that white LED lights, which are increasingly used on a large scale as streetlighting and other outdoor lighting, have strong negative impacts on the mate finding success of glow-worms, even at low light levels. We have furthermore demonstrated that colour temperature does not mitigate the lowered mate attraction success of dummy females under white light.

Keywords

Lampyris noctiluca, glow-worms, light pollution, white light, mate finding, LED.

Introduction

Light of anthropogenic origin has been recognized as an important threat to biodiversity and has been shown to have major impacts on nocturnal wildlife (Hölker et al. 2010a; Hölker et al. 2010b). Artificial light at night (ALAN) produced by streetlights, path lights, illuminated billboards, garden lights, vehicle headlights *etc.* occurs at a worldwide level, and is increasing as the human population, industrial development and urban areas are growing (Hölker et al. 2010a). Currently the nocturnal illumination landscape is shifting from mostly monochromatic streetlighting such as Low Pressure Sodium (LPS) lamps to white LED streetlighting (Elvidge et al. 2010). LED lamps have a broader emission spectrum, allowing a better colour rendering for humans, and emit a larger proportion of blue light compared to the sodium lamps (Davies et al. 2013; Elvidge et al. 2010; Gaston et al. 2012). Because of their relatively high energy impact, short wavelengths can damage vulnerable structures of animal eyes (Contín et al. 2016; Tosini et al. 2016). Moreover, blue light inhibits the production of the hormone melatonin (Tan et al. 2010), which interferes with biological rhythms and by extension health and overall fitness. This has been shown in a wide range of organisms (Bayarri et al. 2002; Csernus et al. 1999; Nakane et al. 2019; Oliveira et al. 2007; Roenneberg and Hastings 1988; Vera et al. 2010;

Yadav et al. 2015), including humans (Lucas et al. 2014). As worldwide more than 60% of invertebrates and around 30% of vertebrates are nocturnal (Hölker et al. 2010b), ALAN may affect an important number of species and eventually entire ecosystems (Owens and Lewis 2018). Nocturnal and dusk active animals, as well as animals communicating through light signals such as fireflies (beetles belonging to the family Lampyridae) may particularly be affected by this emerging threat. An example of this is the common European glow-worm (*Lampyris noctiluca* L.) (Elgert et al. 2020; Longcore and Rich 2004; Owens and Lewis 2018). Flying males of this widespread glow-worm species search visually for the flightless females which use a bioluminescent light organ to signal their presence (Tyler 2002).

The impacts of ALAN on nocturnal wildlife are receiving increased attention and the body of literature is increasing (e.g. Gaston et al. 2013; Hölker et al. 2010b; Longcore and Rich 2004; Owens et al. 2020; Rich and Longcore 2013). For glow-worms, however, studies on the effects on ALAN are less numerous**.** As the old, often monochromatic streetlights are currently being replaced by broad spectrum LED streetlights (Elvidge et al. 2010; Gaston et al. 2012), and as the quantity of nocturnal light pollution is increasing (Hölker et al. 2010a), it is of great importance to evaluate the exact impacts of this emerging threat on glow-worms and on their populations. It is believed that light pollution is an important driver of population declines for North American species (Fallon et al. 2019; Firebaugh and Haynes 2016) but this is less clear for the common European glow-worm.

Nonetheless, a few studies, both observational and experimental, have already shown that ALAN of different light types may interfere negatively with mate finding of male glow-worms. Ineichen and Rüttimann (2012) observed more glowing females under High Pressure Sodium (HPS) streetlights compared to non-illuminated areas, suggesting lower female mating success. Using female mimicking traps, they furthermore captured no males in these illuminated areas, compared to the dark areas between the streetlamps. In line with this, Stewart et al. (2020) and

Bird and Parker (2014) showed that simulated females near a horizontally positioned streetlighting-simulating light source (a Solaris Megastar™ SLA24A/h lamp at 2.75 m height) and an upward facing white light source (a filament bulb torch) attracted few or no males. Elgert et al. (2020) also showed lower female mate attraction to simulated females located inside versus outside the light cone of a downward facing white LED lamp. These studies mostly provided insights regarding light intensity. However, multiple factors must be taken into account in the assessment of the impacts of artificial light on behaviour or fitness such as intensity, spectral distribution and direction (Elvidge et al. 2010). Short wavelengths (blue light) are for example known to be detrimental for multiple species (Gaston et al. 2013; Gaston et al. 2012; Spoelstra et al. 2017). Light-attracted insects are excessively attracted to short wavelengths (Donners et al. 2018; van Langevelde et al. 2011), which may lead to severe mortality (Owens et al. 2020; Owens and Lewis 2018). Firebaugh and Haynes (2019) found that bright light from a cold white LED reduced the flash rate of the dark-active firefly *Photuris versicolor* by 69.69 % and twilight active male *Photinus pyralis* fireflies to 75%, whereas the flash rate of tethered *P. pyralis* females was reduced to 40% (Firebaugh and Haynes 2016). For common glow-worms, a few studies have suggested that light colour plays a role in their behavioural responses to artificial light. However, Booth et al. (2004) found that shorter wavelengths incorporated in a simulated female light signal reduced the attractiveness of the signal. On the other hand, incorporating long wavelengths (red light) in a simulated female light signal seemed to have a neutral effect on the attractiveness of the signals on males (Booth et al. 2004). What is more, LPS streetlights emitting monochromatic long wavelengths even appeared to attract males as numerous males were found sitting in the illuminated areas (Bek 2015 (unpublished thesis)). It is clear that both the spectral composition and the intensity of artificial lights are of great importance in the assessment of the impacts of ALAN on glow-worms. However, no studies have simultaneously examined the effects of different light characteristics.

In this study we aimed to evaluate the effect of white LED light with different intensities and spectral compositions on the ability of male glow-worms to locate females. We used four white LED light types (two light intensities combined with two colour temperatures) pointing vertically to the sky, from a height of 50 cm, in combination with traps with a LED simulating a glowing female ("female dummy") at varying distances from the light source. This experiment is similar to the setups used by Bird and Parker (2014) and Stewart et al. (2020), who used different distances between traps and different light sources, but only a single light type in each study. Also, we conducted the experiment over a full flight period with sufficient replications (480 traps in total) to achieve sufficient statistical power to disentangle effects of light intensity, colour and distance. Our set-up is particularly relevant to evaluate the impact of commonly used garden lighting. Outdoor lighting can have all kinds of orientations, intensities and sizes, and the use of LEDs is a growing trend worldwide (Allied Market Research n.d.; Schulte-Römer et al. 2019). It has been suggested that the males' yellow pigments in the eye are positioned such that they protect them in particular from light coming from the sky (Booth et al. 2004). Thus, upward oriented garden lighting may have a particularly strong impact on male behaviour. Based on the literature we expect that males are more strongly disturbed in their mate-finding by lights containing a larger proportion of blue light, as well as by more intense lights.

Material and methods

Study area

The study was carried out in June and July 2019 in a forested area (Lippelobos; 51°02'09.7"N 4°14'53.0"E) near Lippelo, Belgium, where glow-worms were known to occur in high densities. The site consisted of a mixed deciduous forest, mostly dominated by beech (*Fagus* sp.) and chestnut (*Castanea* sp.) trees with little undergrowth.

Light-lure traps

Males were trapped with custom-made light-lure traps constructed from opaque plastic bottles with the bottle neck cut off and flipped to function as a funnel. The traps had a diameter of 8.5 cm and a height of approximately 15 cm. Sewing thread was inserted in the bottle opening to prevent males from escaping. At the top of the bottle, a rectangular diffuse lime green LED light (λ_{max} = 555 nm, 6 mcd, 20 mA) was mounted, imitating a female glow-worm's lantern in both intensity and peak wavelength (Bird and Parker 2014; Booth et al. 2004; De Cock 2004; Hopkins et al. 2015; Schwalb 1960). The light circuits were powered by two AA batteries of 1,5V which were placed on the bottom of the trap. Resistors were used to obtain the desired light intensity with a mean resistance of 612 Ω, (SD = 1.41 Ω; N = 61). The light intensity varied between 0.49 and 1.00 lux with an average of 0.79 lux (SD = 0.13 lux, N = 75 traps). This corresponds with the average glow intensities calculated on the basis of female lantern surface and intensity data provided by Booth et al. (2004) and Hopkins et al. (2015). The light intensity was measured with a luxmeter (Skye® SKL 310), with the sensor oriented towards the tip of the LED, keeping 1 cm between them.

Experimental setup

Traps were placed linearly at a distance of 0 m, 1 m, 2 m, 5 m, 10 m and 20 m from a white LED light source (Fig. 1). For this purpose, eight linear patches of approximately 20 meters in length were selected within an area of c. 700 m in diameter. Patches were mostly free of undergrowth, thus the visibility of the white light was unobstructed throughout the patch. None of the patches were influenced by intrusive light from streetlighting or by the treatments in other patches. Two patches were located in an area dominated by chestnut trees and smaller chestnut saplings with a thick litter layer. Three were in an area with large beech trees without undergrowth, also with a leaf litter layer. One patch was situated in a more densely wooded part with an undergrowth of bramble (*Rubus* sp.). A seventh patch was on the edge of a grassy unpaved road crossing the beech woods. The last patch was in a dry small stream bedding surrounded by chestnut trees and saplings at one side and a young and more dense beech stand at the other side. Each night, one patch was arbitrarily selected for each of the five treatments described below, and three patches were left unused. The treatments were arbitrarily assigned to patches, except that the same treatment was not used on two consecutive nights in the same patch, and the number of control treatment was kept more or less constant across the patches. Between nights, the position of the white light was arbitrarily switched between the two ends of the patch. Each night we randomly selected 30 out of the 75 available traps to be used in the experiment and the identity of all traps was noted.

In each treatment, one of four different types of LED light was used. This artificial light source consisted of one or eight white LEDs soldered on a small electrical circuit powered by two or four batteries, and taped on top of a wooden stick of 50 cm height, positioned at distance zero (Fig. 1). The light was pointed vertically to the sky and no armature or shielding was used, thus allowing the light to spread in all directions. Two treatments consisted of warm white LEDs of 22 000 mcd (20 mA, 3,2V): the light source of weak intensity consisted of only one white LED, powered by two AA batteries of 1.5V and the strong intensity consisted of eight white LEDs, powered by four AA batteries of 1.5V. The same was done with cold white LEDs of 22 000 mcd (20mA, 3,6V). All the LEDs were covered with tracing paper with the purpose of creating more diffuse light sources. The fifth treatment was a control without white LED lights. The light intensities of the treatments at the different distances are reported in Fig. S1. Supplementary material.

Traps were placed around sunset (10.00 PM) and removed around 01:00 AM, to cover the male flight period which is estimated to occur between 10.00 PM and 12.00 AM (Bird and Parker 2014; Ineichen and Rüttimann 2012). After being counted, the males were released at about 5 to 20m from the patch where they were caught . The experiment was set up during 16 nights with dry weather conditions between the 26th of June and the 13th of July 2019 which covered the entire male flight season as judged from the numbers of males caught (Fig. S2. Supplementary material). On the 10th and 12th of July this experiment was not set up due to rain.

Statistical analysis

The statistical analysis was performed using R (2019). A Linear-Mixed-Effects (LME) model was used to account for the repeated measurements on the same patches, using the "lmerTest" package (Kuznetsova et al. 2017). We discarded nights with fewer than 30 males caught in total, i.e. less than one per trap on average, to avoid overdispersion due to an excess of null values. The statistical analysis was thus based on data of 12 experimental nights, between 28th of June and the 9th of July. The number of males caught in the traps was taken as response variable. The logarithm (+1) was taken as this improved the normality of the distribution of the residuals. The dates were converted into Julian dates and centered around the mean. Distance was converted to the natural logarithm of (distance + 0.75) since it rendered a better model fit and a better visual graphical representation. Treatment, date, date squared, distance, and distance squared were included in the model as fixed effects. The date and date squared were added to represent the bell-shaped curve of male abundance which is typical for the short flight season. We included both distance and distance squared to explore non-linear effects of the treatment on male attraction success. We also included the interaction between treatment and distance (as well as squared distance) to test whether the effect of the white lights varied as a function of the distance. The trap identity and patch identity were included as random effects. The model fit was evaluated with the Bayesian Information Criterion (BIC). Residuals were normally distributed (Shapiro Wilk normality test, $W = 0.99$, $P = 0.15$).

In an additional model, we evaluated if the treatment effects were mostly due to light intensity (weak/intense) or colour temperature (cold/warm white). We did this by comparing the BIC of three models whereby treatments were pooled in different ways, leaving out the control treatment. The first model considered the four treatments separately. The second model pooled the data across the different colour temperatures and consisted of two groups: the intense light treatments and the weak light treatment. The third model pooled the data across light intensities and had two groups based on colour temperature.

Results

In total exactly 1000 male glow-worms were caught in 479 traps (30 light traps/night excluding one trap that fell over and was not included in the data). Sixty-one percent of the traps contained at least one male, with a maximum of 25 in one trap. Fig. S2 in Supplementary material shows the number of males caught each night.

The LME model confirmed that the number of caught males varied significantly over time with a quadratic relationship (Table 1). The model also showed two-way interactions between treatment and distance, both the linear and quadratic components (Table 1). Fig. 2 shows the number of males caught at the different distances in each treatment, as well as the model predictions. In all treatments, significantly more males were captured at greatest distances from the light source. This was also the case in the control treatment, although here the difference was less pronounced, showing a J shaped relationship with distance. Furthermore, all light treatments had significantly fewer males in their traps compared to the control treatment, but this difference decreased with distance (Fig. 2). Next, under weak light intensities the number of males was lower compared to the control treatment, especially at the smaller distances from the light source. At larger distances the number of caught males increased quite similarly to the control treatment. Finally, under strong light intensities, the number of males were very low in the more proximal traps but increased towards the rear end of the transect, resulting in a non-linear relationship (Fig. 2).

In the last step, we compared the BIC values of three models with differently pooled data to determine which factors described the data best. When we compared the models with either the four treatments (BIC = 600.54), the treatments grouped by light intensity (BIC = 556.80) and grouped by colour temperature (BIC = 607.32), the model with the pooled data according to light intensity clearly had the lowest BIC value with similar effects of treatment, including interactions with distance ($P < 0.05$) (Table S1 in Supplementary material). Fig. 3 shows the predictions of the model with only light intensity, showing significantly lower number of males under intense light treatments, in particular at relatively short distances to the light source (1 to 5m).

Discussion

Our experiment with multiple light treatments and a large number of replicates carried out over the entire flight season allowed us to confirm that white LED light has considerable negative effects on mate-finding by male glow-worms. This confirms suggestions from earlier studies using different sources of white light (Bird and Parker 2014; Elgert et al. 2020; Stewart et al. 2020). In our study we extended their approach using more traps over a larger intensity and distance gradient and with extensive replication, allowing us to compare the response to two light intensities (weak, strong), combined with two colour temperatures (cold, warm). Our results clearly show that the intensity of the white LED-lights is the main factor impacting female attraction success, as male capture rates were significantly lower in the intense light treatment over most of the range of distances. Although Fig. 2 could suggest that the warmer colour temperatures of white LEDs yielded slightly higher male capture rates than the colder colour temperatures, the model including temperature performed considerably less well than the model with only light intensity, despite the strictly controlled comparison and large sample size. We should note that the warm white LEDs were also somewhat less intense than the cold white LEDs, which led to substantially lower intensities measured at 0m (25% and 60% difference for intense and weak treatments) but much smaller differences at 1m and beyond (Fig. S1). Nevertheless, especially at close range (<1m) and with the highest intensity, the warm light did not attract more males than the cold light. The two white LED types differed quite considerably in their spectral emission composition, with a dominant short-wavelength emission peak in cold white, and a dominant peak in long wavelengths in the warm white LEDs (Fig. S3 Supplementary material). Pawson and Bader (2014) studied the effects of white LEDs on nocturnal invertebrates by counting the number of invertebrates attracted to HPS or white LED lights. They also found no difference between colour temperatures, suggesting that changing the wavelength composition of the white LED streetlighting will not mitigate their ecological impacts. As short wavelengths were shown to decrease the attractiveness of female signals (Booth et al. 2004), and with the knowledge that only two expressed opsin classes have been found in other firefly species, one in the ultraviolet-sensitive and one in the long-wavelength-sensitive areas of the visible spectrum (the long wavelength mechanism in close tune with the species bioluminescence emission spectrum) (Lall et al. 1980; Martin et al. 2015), we predicted that cold white light would induce a lower capture rate of the traps. Our results however showed that the difference in colour temperature had no impact on the capture rate. So despite the lower proportion of short wavelengths in the warm white LEDs, they do not seem to form an eco-friendly alternative for street and outdoor lighting dominated by short wavelengths in their emission spectrum, with respect to glow-worms. We used our data to evaluate the minimal threshold intensity at which white LED light starts to have a negative impact on the males' ability to locate females, by looking at the shortest distance (and corresponding light intensity) where the error bars of control and each light treatment start to overlap (Fig. S4. Supplementary material). This resulted in threshold values of 0.052 lux (intense warm white), 0.013 lux (intense cold white), 0.028 lux (weak warm white) and 0.014 lux (weak cold white), which corresponds to an average of 0.027 lux. This is a mere 0.017 lux above the average light intensity measured in the control treatment without any light added. Note that this can be considered a conservative estimate, since with an even larger sample size and a light gradient with more traps we might reach an even lower and more fine-tuned threshold value. According to what we found in the literature, our results showed the lowest intensity threshold value ever reported to negatively impact glow-worm mate-seeking abilities (Bird and Parker 2014; Ineichen and Rüttimann 2012). Yet, this also probably depends on the wavelength composition and orientation of the light source being studied. Bird and Parker (2014) found a threshold of 0.09 lux above which they found significantly less males in the traps, using an upward facing light. Upward facing lights, such as certain garden lighting or other outdoor lighting types, are thought to have a greater desensitisation (dazzling) effect compared to downward facing lights when males fly above them, due to the fronto-dorsal distribution of blue-filtering pigments in the male eyes (Booth et al. 2004). This could contribute to the very low threshold values at which the males experience negative impacts of light pollution found in our study. Stewart et al. (2020) used a horizontally oriented white light and found significant differences in male attraction up to 55 m compared to the dark control treatment, which makes it not possible to compare these results with our light intensity threshold. More research is needed to verify whether downward facing white LED lights may have lower impact on glow-worms.

Several underlying mechanisms could be responsible for the lower capture rates close to white LED light. In the case of the response of glow-worms to white light, we could expect four mechanisms to occur : (i) a desensitisation/dazzling effect (Owens and Lewis 2018), (ii) repulsion (Schwalb 1960), (iii) a wash-out effect (Longcore and Rich 2004) or, (iv) mate-seeking behaviour inhibition (Booth et al. 2004). Desensitisation can be described as an excessive stimulation by too many photons at once of the highly sensitive visual system of nocturnal insects. This may cause temporary dazzling or permanent blinding of some insects (Owens and Lewis 2018). Firstly, desensitisation induced by bright artificial light has been observed in *Photinus* fireflies, which translates into an increased time needed to adapt their eyes to the dark after being exposed to a bright light source (Lall 1993). Secondly, repulsion corresponds to negative phototaxis induced by artificial light. Negative phototaxis from an artificial light source has been reported in *L. noctiluca* by Schwalb (1960) at high light levels (from 500 lux). Thirdly, the wash-out effect affects the ability of insects to recognise objects in their surroundings by reducing the contrast between a light signal and the background (Longcore and Rich 2004). Light of different wavelengths can enhance or reduce the ability of nocturnal insects to discriminate colours and objects (Davies et al. 2013). Glow-worms, as well as many other Lampyrids, are known to have two types of photoreceptors, one with a peak sensitivity coinciding with the spectral emission of the female, and one with a peak sensitivity coinciding with short wavelengths (blue and UV-light) (Booth et al. 2004; Lall et al. 1980; Martin et al. 2015). It can thus be assumed that males would not be able to discriminate between green and yellow/red lights for example, as they are both characterised by long wavelengths. Finally, Booth et al. (2004) observed that males showed a reduced attraction towards a green light stimulus when combined with blue light. The setup of our experiment does not allow us to discriminate between these hypotheses. It is also probable that a combination of mechanisms causes the lowered ability of the males to locate the females due to white light pollution. It can be assumed that these mechanisms differ in function of the spectral composition and intensity of the light. More specific experiments will be needed to further elucidate these questions.

To our surprise, the control treatment showed an unexpected J-shaped relationship between the number of males and the distance from the light source rather than a uniform distribution of males over the traps. We can exclude that this pattern is a by-product of how males were released after capture, since they were scattered in the study area; furthermore, the orientation of trap lines and treatments were randomly alternated between experimental nights. We can also rule out biases due to the specific location of the trap lines. While males can be expected to follow edges of open spaces or forests since females are known to prefer this kind of habitat as a display site (Atkins et al. 2016), all (except one) trap lines were away from forest edges nor did they include open spaces. Thus, we can rule out male flight preferences as explanation for the observed distribution pattern. We rather propose that the J-shaped pattern in the control treatment is due to the specific aggregation pattern of traps in each patch, whereby traps were clumped closest to the light source, or at the corresponding proximal end of the control treatment. In a hypothetical scenario where males approach the patch from random directions, are uniformly distributed and are attracted to the closest female dummy they encounter, we can expect that more individuals will be found at both of the extremities of the set of traps (Fig. 4). Even if males are not attracted to the nearest dummy female, the specific trapline pattern may still generate spatial biases, for example if males fly around to inspect the different simulated females, and as a consequence tend to linger at the extremities of the trap line. Hopkins et al. (2015) confirmed that males do not simply go to the closest female they encounter but that their mate choice is based on female brightness which is correlated to female fecundity. However, when females are not spatially clumped their relative brightness is no longer important (Borshagovski et al. 2018). The recent study of Stewart et al. (2020) similarly found more males in the last trap of a linear setup, irrespective of the exact length of the transect. They also suggested that the distribution of captured males is due to a combination of reduced competition from a neighboring trap at the terminal position, and/or the fact that males may usually stop at the first trap they encounter. Whatever the explanation, the pattern in the control treatment has no implications whatsoever for our general conclusions, but it shows how trap set-up may strongly influence overall outcomes, and highlights the importance of a proper control, as well as randomization of trap-line orientations.

In conclusion, we show that upward-facing LED lights – as increasingly used in garden lighting – lead to a dramatic decrease in attraction of male glow-worms to females, thus lowering female mating success. This effect was seen even at low light levels of 0.027 lux on average and at distances up to 5 to 10 m for our intense light treatments and 1 m to 2 m for the weak light treatments. The ongoing trend of replacing old streetlighting by white LEDs and the general increase in ALAN do not forecast a favorable view of the future for glow-worm populations. Indeed, since white light strongly lowers the mate-attraction success of the females even at very low light levels, in combination with the recent finding of Elgert et al. (2020) that female glow-worms do not mitigate this effect by moving away from artificial white light, it is clear that white light pollution threatens glow-worm populations mostly located closer to urbanised areas. This negative impact has been confirmed by observations and experiments showing that females may remain unmated for long periods and even do not mate at all due to ALAN (Bird and Parker 2014; Elgert et al. 2020; Ineichen and Rüttimann 2012; Van den Broeck et al. in prep.). Interestingly, our results in a forested area are highly similar to those obtained by Stewart et al. (2020) in more open habitat. This confirms that the observed effects of ALAN on glow-worm mating can be generalized across different habitats. We also showed that using white lights of different colour temperature does not mitigate the negative effects of the white lights on glow-worms. We thus advise against the placement of white LED streetlights and white LED outdoor lighting in potential glow-worm habitat. This is in line with the majority of the recommendations proposed by studies on nocturnal insects and bats suggesting to avoid broad spectrum lights (Fallon et al. 2019; Gaston et al. 2012; Owens et al. 2018; Spoelstra et al. 2017; van Langevelde et al. 2011). The intensity of artificial light and its impact on wildlife deserve more scientific attention than it has received to date, especially given the increasing brightness of outdoor illumination in general and by LEDs in particular.

References

Allied Market Research (n.d.) Outdoor Lighting Market Outlook - 2026. Accessed on 29 October 2020, available online at: https://www.alliedmarketresearch.com/outdoor-lighting-market.

Atkins V et al. (2016) The status of the glow-worm *Lampyris noctiluca* L. (Coleoptera: Lampyridae) in England 4:20-35

Bayarri M, Madrid J, Sánchez-Vázquez F (2002) Influence of light intensity, spectrum and orientation on sea bass plasma and ocular melatonin Journal of pineal research 32:34-40

Bird S, Parker J (2014) Low levels of light pollution may block the ability of male glow-worms (*Lampyris noctiluca* L.) to locate females Journal of insect conservation 18:737-743

Booth D, Stewart AJ, Osorio D (2004) Colour vision in the glow-worm *Lampyris noctiluca* (L.)(Coleoptera: Lampyridae): evidence for a green-blue chromatic mechanism. Journal of Experimental Biology 207:2373-2378

Borshagovski A-M, Baudry G, Hopkins J, Kaitala A (2018) Pale by comparison: competitive interactions between signaling female glow-worms Behavioral Ecology 30:20-26

Contín M, Benedetto M, Quinteros-Quintana M, Guido M (2016) Light pollution: the possible consequences of excessive illumination on retina Eye 30:255-263

Csernus V, Becher P, Mess B (1999) Wavelength dependency of light-induced changes in rhythmic melatonin secretion from chicken pineal gland *in vitro* Neuroendocrinology Letters 20:299-304

Davies TW, Bennie J, Inger R, De Ibarra NH, Gaston KJ (2013) Artificial light pollution: are shifting spectral signatures changing the balance of species interactions? Global change biology 19:1417-1423

De Cock R (2004) Larval and adult emission spectra of bioluminescence in three European firefly species. Photochemistry and photobiology 79:339-342

Donners M, van Grunsven RH, Groenendijk D, van Langevelde F, Bikker JW, Longcore T, Veenendaal E (2018) Colors of attraction: Modeling insect flight to light behavior Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 329:434-440

Elgert C, Hopkins J, Kaitala A, Candolin U (2020) Reproduction under light pollution: maladaptive response to spatial variation in artificial light in a glow-worm Proceedings of the Royal Society B 287:20200806

Elvidge CD, Keith DM, Tuttle BT, Baugh KE (2010) Spectral identification of lighting type and character Sensors 10:3961-3988

Fallon C, Hoyle S, Lewis S, Owens A, Lee-Mäder E, Black SH, Jepsen S (2019) Conserving the jewels of the night: guidelines for protecting fireflies in the United States and Canada. The Xerces Society For Invertebrate Conservation, Portland, Oregon

Firebaugh A, Haynes KJ (2016) Experimental tests of light-pollution impacts on nocturnal insect courtship and dispersal Oecologia 182:1203-1211

Firebaugh A, Haynes KJ (2019) Light pollution may create demographic traps for nocturnal insects Basic and Applied Ecology 34:118-125

Gaston KJ, Bennie J, Davies TW, Hopkins J (2013) The ecological impacts of nighttime light pollution: a mechanistic appraisal Biological reviews 88:912-927

Gaston KJ, Davies TW, Bennie J, Hopkins J (2012) Reducing the ecological consequences of night-time light pollution: options and developments Journal of Applied Ecology 49:1256-1266

Hölker F et al. (2010a) The dark side of light: a transdisciplinary research agenda for light pollution policy Ecology and Society 15

Hölker F, Wolter C, Perkin EK, Tockner K (2010b) Light pollution as a biodiversity threat Trends in ecology & evolution 25:681-682

Hopkins J, Baudry G, Candolin U, Kaitala A (2015) I'm sexy and I glow it: female ornamentation in a nocturnal capital breeder Biology letters 11:20150599

Ineichen S, Rüttimann B (2012) Impact of artificial light on the distribution of the common European glow-worm, *Lampyris noctiluca* (Coleoptera: Lampyridae) Lampyrid 2:31-36

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest package: tests in linear mixed effects models Journal of statistical software 82

Lall AB (1993) Action spectra for the initiation of bioluminescent flashing activity in males of twilight-active firefly *Photinus scintillans* (Coleoptera: Lampyridae) Journal of insect physiology 39:123-127

Lall AB, Chapman RM, Trouth CO, Holloway JA (1980) Spectral mechanisms of the compound eye in the firefly *Photinus pyralis* (Coleoptera: Lampyridae) Journal of comparative physiology 135:21-27

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

Lucas RJ et al. (2014) Measuring and using light in the melanopsin age Trends in neurosciences 37:1-9

Martin GJ, Lord NP, Branham MA, Bybee SM (2015) Review of the firefly visual system (Coleoptera: Lampyridae) and evolution of the opsin genes underlying color vision Organisms Diversity & Evolution 15:513-526

Nakane Y et al. (2019) Action spectrum for photoperiodic control of thyroid-stimulating hormone in Japanese quail (*Coturnix japonica*) PloS one 14:e0222106

Oliveira C, Ortega A, López-Olmeda JF, Vera LM, Sánchez-Vázquez FJ (2007) Influence of constant light and darkness, light intensity, and light spectrum on plasma melatonin rhythms in Senegal sole Chronobiology international 24:615-627

Owens A, Cochard P, Durrant J, Farnworth B, Perkin E, Seymoure B (2020) Light pollution is a driver of insect declines Biological Conservation 241:108259

Owens A, Lewis SM (2018) The impact of artificial light at night on nocturnal insects: A review and synthesis Ecology and evolution 8:11337-11358

Owens A, Meyer-Rochow VB, Yang E-C (2018) Short-and mid-wavelength artificial light influences the flash signals of *Aquatica ficta* fireflies (Coleoptera: Lampyridae) PloS one 13:e0191576

Pawson SM, Bader M-F (2014) LED lighting increases the ecological impact of light pollution irrespective of color temperature Ecological Applications 24:1561-1568

R Core Team (2019) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Rich C, Longcore T (2013) Ecological consequences of artificial night lighting. Island Press, Washington

Roenneberg T, Hastings J (1988) Two photoreceptors control the circadian clock of a unicellular alga Naturwissenschaften 75:206-207

Schulte-Römer N, Meier J, Söding M, Dannemann E (2019) The LED Paradox: How light pollution challenges experts to reconsider sustainable lighting Sustainability 11:6160

Schwalb HH (1960) Beiträge zur Biologie der einheimischen Lampyriden *Lampyris noctiluca* Geoffr. und *Phausis splendidula* Lec. und experimentelle Analyse ihres Beuteumfang-und Sexualverhaltens. Verlag nicht ermittelbar

Spoelstra K et al. (2017) Response of bats to light with different spectra: light-shy and agile bat presence is affected by white and green, but not red light Proceedings of the Royal Society B: Biological Sciences 284:20170075

Stewart AJ, Perl CD, Niven JE (2020) Artificial lighting impairs mate attraction in a nocturnal capital breeder Journal of Experimental Biology 223

Tan DX et al. (2010) The changing biological roles of melatonin during evolution: from an antioxidant to signals of darkness, sexual selection and fitness Biological Reviews 85:607-623

Tosini G, Ferguson I, Tsubota K (2016) Effects of blue light on the circadian system and eye physiology Molecular vision 22:61

Tyler J (2002) The Glow-worm. Sevenoaks: Lakeside Printing Ltd.,

van Langevelde F, Ettema JA, Donners M, WallisDeVries MF, Groenendijk D (2011) Effect of spectral composition of artificial light on the attraction of moths Biological conservation 144:2274-2281

Vera L, Davie A, Taylor J, Migaud H (2010) Differential light intensity and spectral sensitivities of Atlantic salmon, European sea bass and Atlantic cod pineal glands *ex vivo* General and comparative endocrinology 165:25-33

Yadav G, Malik S, Rani S, Kumar V (2015) Role of light wavelengths in synchronization of circadian physiology in songbirds Physiology & behavior 140:164-171

Tables and figures

The figures were made with the following programs: R, Microsoft PowerPoint or MatLab.

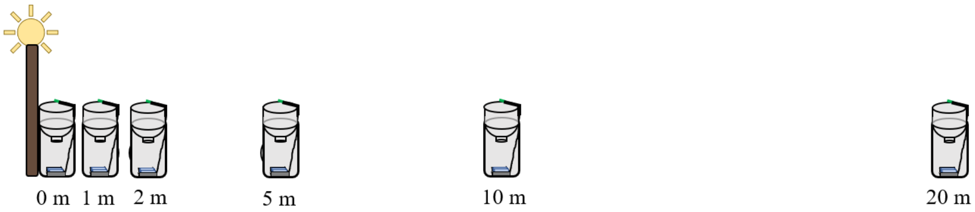


Fig. 1 Schematic overview of the experimental setup with a LED light positioned on the left, and traps placed at different distances. Note that the traps and the light source are not to scale

Table 1 Results of the LME full model on variation on in males caught per trap in relation to the five treatments, distance and date. Note that Julian dates were centered around the mean. Significant effects are indicated in bold (*= *P*<0,05; **= *P*<0,01; ***= *P*<0,001)

Fixed effects	**F value**	**Num DF**	**Den DF**
Julian date	0.0014	1	325.51
(Julian date)^2	**43.93** ***	1	306.11
Log(distance)	1.27	1	330.06
Log(distance)^2	**43.93** ***	4	331.11
Treatment	**9.78** ***	4	334.79
Treatment × log(distance)	**6.26** ***	4	332.34
Treatment × (log(distance))^2	**5.77** ***	4	332.19

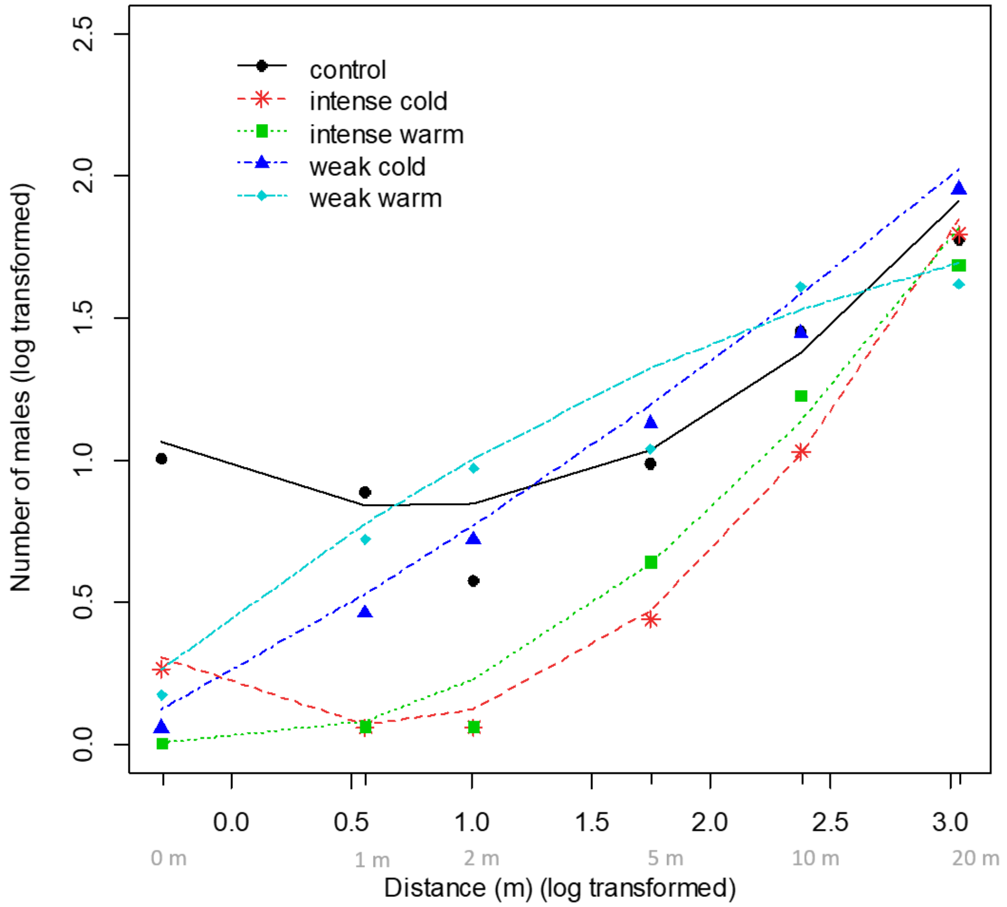


Fig. 2 Graph with the model predictions (lines) and the observed means (symbols) of the number of males caught at different distances for the five treatments. Note the logarithmic scale on both axes. For a better readability, the confidence intervals are not included and the actual distances of the traps from the light source are indicated as minor tick marks in grey

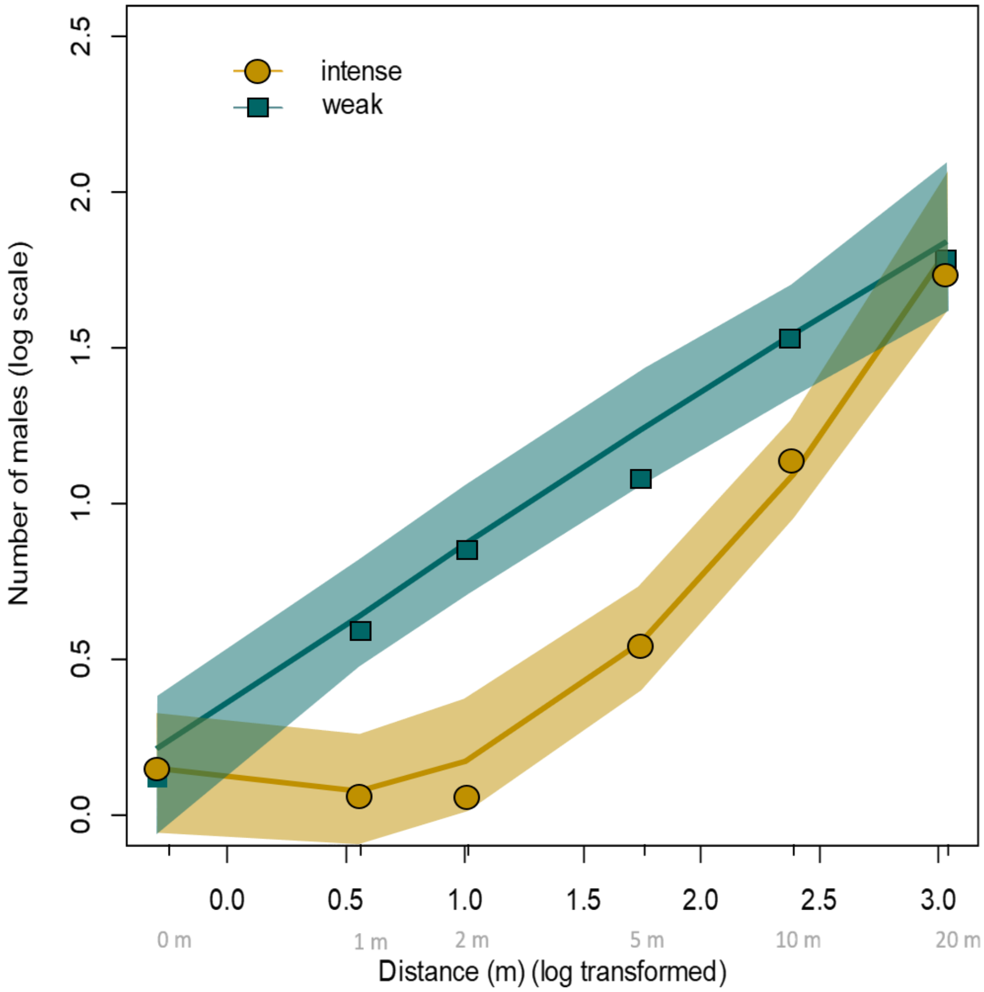


Fig. 3 Graph with the model predictions (lines), the observed mean estimates (symbols), and the 95% confidence intervals (hatched areas) of the model based on the pooled data according to light intensity, regardless of colour temperature. Note the logarithmic scale on both axes

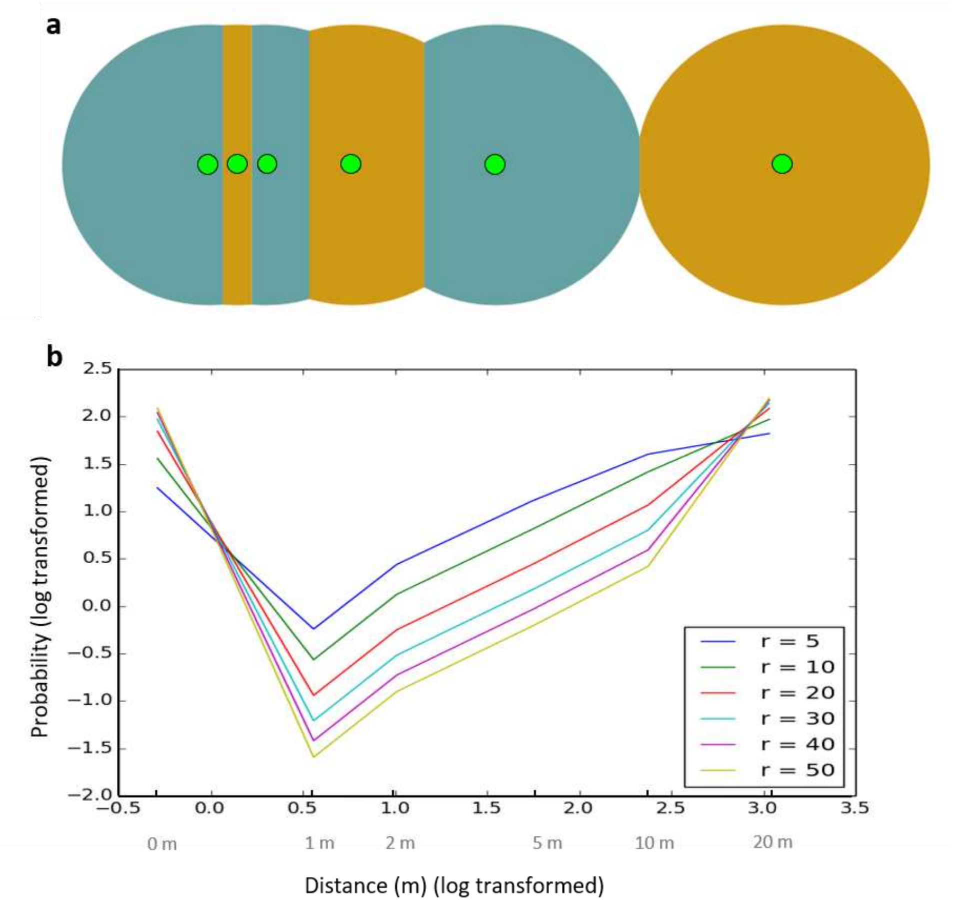


Fig. 4 Visualization of the hypothesis explaining the male distribution in the control treatment. (**a**) Set of traps of the control treatment placed at 0 m, 1 m, 2 m, 5 m, 10 m and 20 m. Circles represent the area from which incoming males are attracted to each trap, assuming they move to the nearest trap they encounter (**b**) Expected distribution of males for different sizes of the circles shown in (a) (r = radius in meter). Longer attraction distances (i.e. larger radii) result in an increasingly J-shaped distribution of males

Supplementary information

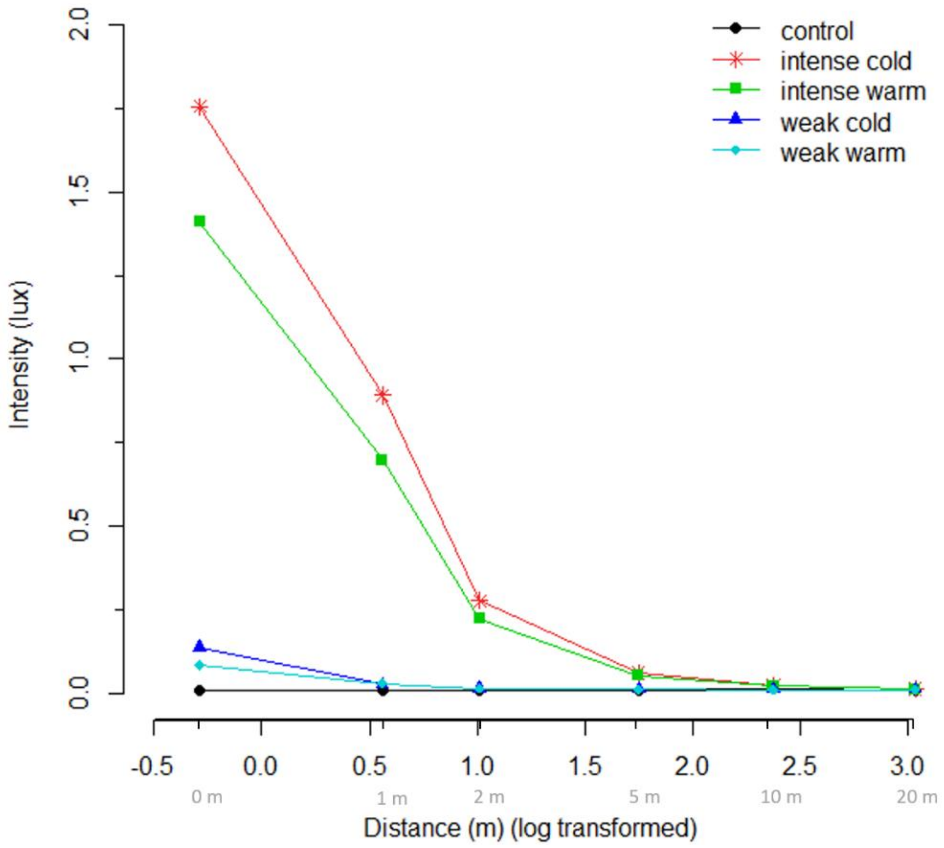


Fig. S1 Light intensities measured at the top of a light trap at 0 m, 1 m, 2 m, 5 m, 10 m and 20 m distance. Note the log scale, with true distances indicated as minor tick marks and in grey for more clarity. Each value is an average of three measurements. The light intensities from the treatments across the transect were measured with the same luxmeter as mentioned before. These latter measurements were carried out in similar conditions as the experiment, in a deciduous forest with no additional artificial lights and with low moonlight conditions on 12 April 2019

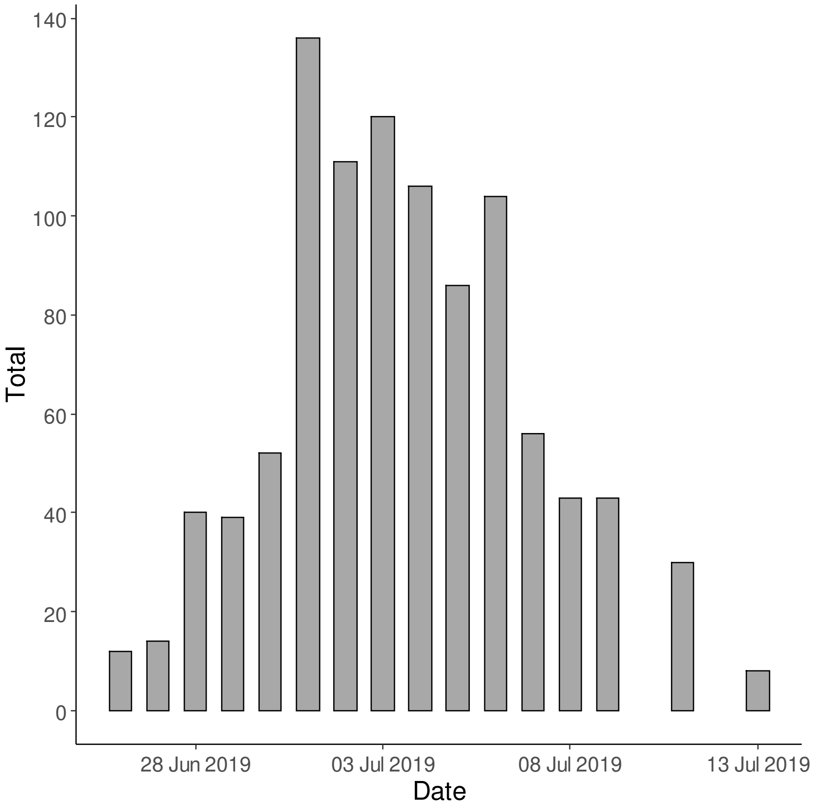
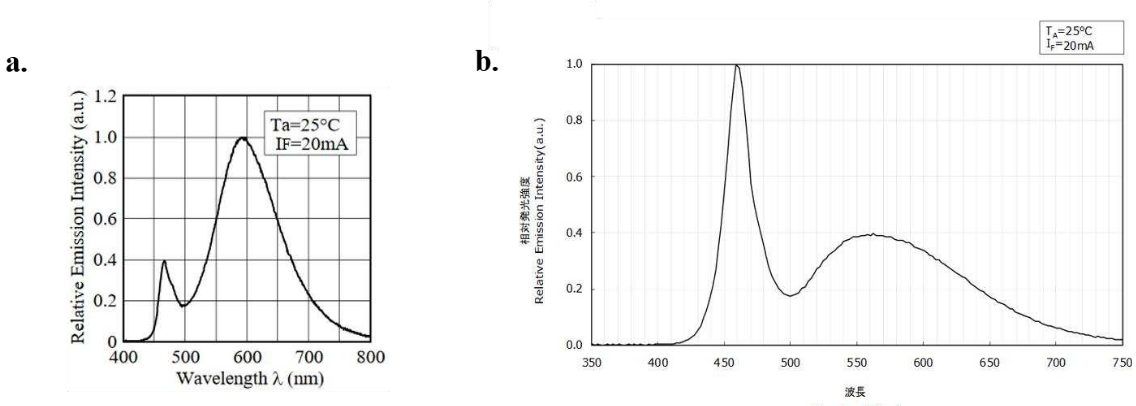


Fig. S2 Number of male glow-worms caught each night on a total of 16 days between 26th of June and 13th of July 2019

Table S1 Results of the LME model on variation in males caught per trap in relation to the pooled data according to light intensity, distance and date. Significant effects are indicated in bold (*= *P*<0,05; **= *P*<0,01; ***= *P*<0,001)

Fixed effects	**Estimate**	**F value**	**Num DF**	**DenDF**
Julian date	-0.0356	1.23	1	273.27
(Julian date)^2	-0.211	**33.59 ***	1	254.10
Log(distance)	0.543	**5.39 ***	1	271.83
Log(distance)^2	-0.0149	**15.69 *****	1	270.67
Treatment	-0.263	**5.66 ***	1	275.52
Treatment × log(distance)	-0.696	**17.33 *****	1	271.90
Treatment × (log(distance))^2	0.255	**20.21 *****	1	271.94



Warm white

Cold white

Fig. S3 Spectra of the white LEDs used in the study, as indicated in the datasheet provided by the seller. **a**. Warm white LEDs (NSPL500DS Sel. F3/5V) and **b**. Cold white LEDs (NSPW500DS)

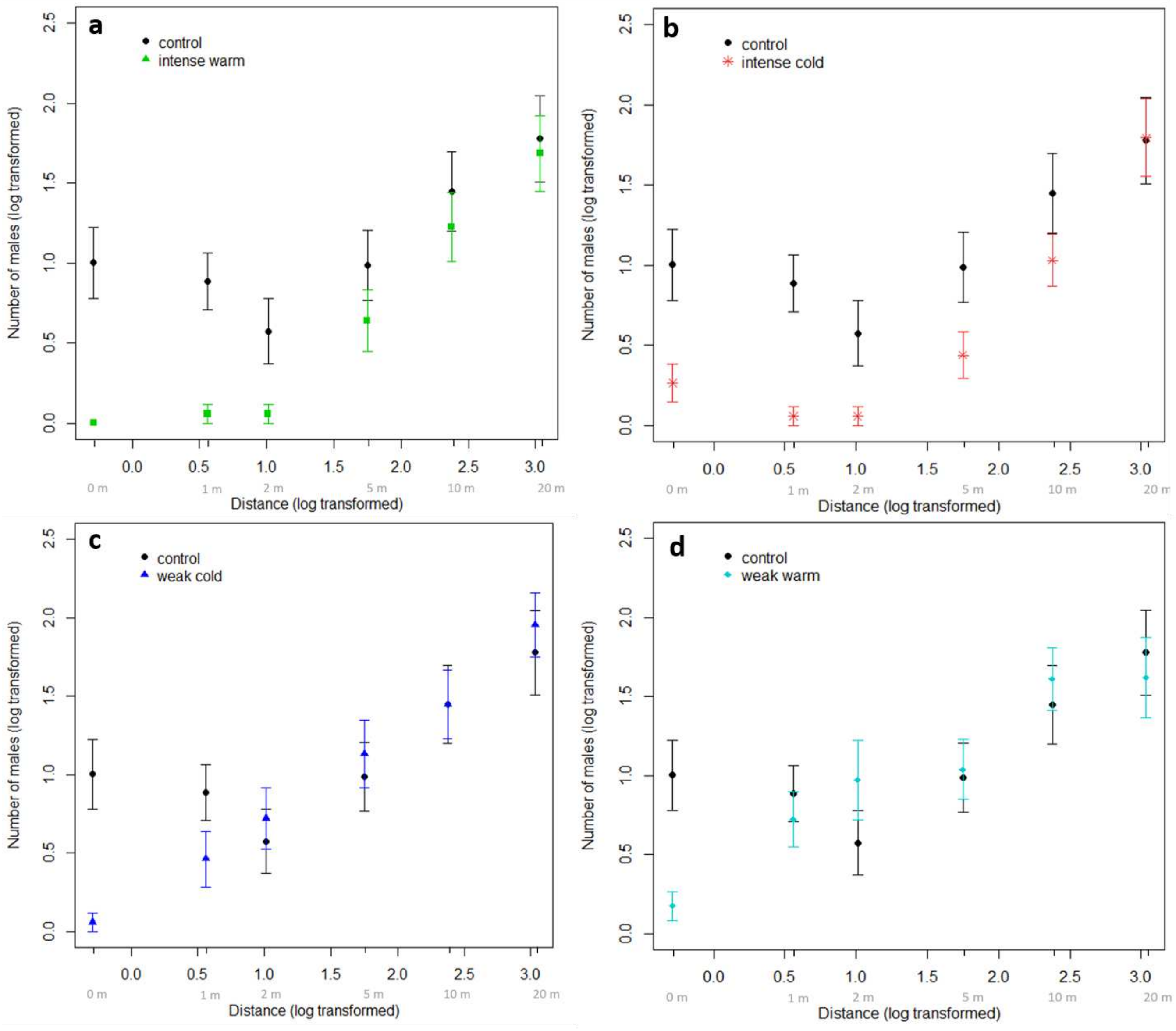


Fig. S4 Graphs with the observed mean estimates (symbols), and the error bars of each treatment versus the control treatment, used to determine the highest light intensity where the treatment no longer differs from the control . **a.** The error bars of the control overlap with those of the intense warm light treatment at 5m (0.052 lux). **b.** The error bars of the control overlap with those of the intense cold light treatment at 20m (0.013 lux). **c.** The error bars of the control overlap with those of the weak cold light treatment at 2m (0.014 lux). **d.** The error bars of the control overlap with those of the weak warm light treatment at 1m (0.028 lux). Note the logarithmic scale on both axes