Effects of lifetime exposure to artificial light at night on cricket (*Teleogryllus commodus*) courtship and mating behaviour

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Increasing evidence suggests that key fitness-related behaviours of animals related to courtship and mating may be disrupted by anthropogenic stressors, including artificial light at night (i.e. light produced from anthropogenic sources). Despite its ubiquity in urban habitats, we currently know very little about how artificial night lighting affects the reproductive behaviours of most animals. Our study examined the effects of chronic (lifetime) exposure to one of four ecologically relevant intensities of artificial light at night (0, 1, 10 or 100 lx at night) on courtship and mating behaviours and acoustic sexual signalling in a common nocturnal and crepuscular insect, the Australian black field cricket, *Teleogryllus commodus*. We found that lifetime exposure to brighter (10–100 lx) artificial light at night affected some aspects of courtship and mating behaviour: it influenced mate choice and mating efficiency in a sex-specific manner, but did not affect the multivariate structure of male courtship calls. Our results suggest that chronic exposure to bright light at night may affect some aspects of mate choice and reproductive behaviour in this common insect, and warrants further study across taxa.

For sexually reproducing animals, successfully attracting and copulating with a mate is paramount for individual fitness and population persistence. Species employ a range of cues to court potential mates, including specific behaviours, visual, chemical and/or acoustic signals, or a combination of different signals (Candolin, 2003). These signals can be affected by a range of external biotic (e.g. parasitism risk: Zuk, Rotenberry, & Tinghitella, 2006) and abiotic factors, such as temperature, light or noise (Endler, 1992). Increasing evidence suggests that anthropogenic stressors can also influence courtship signalling and mating-related behaviours in a variety of animals (Blocker & Ophir, 2013). Chemical contaminants, for example, reduce the olfactory attractiveness and mating success of male palmate newts, *Triturus helveticus* (Secondi, Hinot, Djalout, Sourice, & Jadas-Hécart, 2009), and can impair the expression of sexually selected ornaments, courtship behaviour and reproductive success of two species of fish: the darkedged splitfin, *Girardinichthys multiradiatus* (Arellano-Aguilar & Garcia, 2008) and the guppy, *Poecilia reticulata* (Shenoy, 2012). Similarly, anthropogenic noise pollution is correlated with shifts in acoustic courtship signals, including call rate and frequency in anuran amphibians (Lengagne, 2008; Parris, Velik-Lord, & North, 2009; Sun &

Narins, 2005), orthopteran insects (Lampe, Schmoll, Franzke, & Reinhold, 2012; Schmidt, Morrison, & Kunc, 2014) and birds (Luther, Phillips, & Derryberry, 2016; Patricelli & Blickley, 2006), resulting in reduced attractiveness of the signal, which ultimately has implications for pair bonding, mating success and reproductive fitness (Halfwerk & Slabbekoorn, 2014; Swaddle & Page, 2007). A less well studied pollutant that has enormous potential to affect animals living in urbanized areas is artificial light at night (i.e. light produced from anthropogenic sources).

The presence of artificial light at night is arguably one of the most recent and yet pervasive forms of anthropogenic pollution (Falchi et al., 2016). The majority of species have evolved under a predictable bright day, dark night daily (circadian) cycle and have physiological, ecological and behavioural traits that are optimized for this daily rhythm (Gerrish, Morin, Rivers, & Patrawala, 2009; Kronfeld-Schor & Dayan, 2003; Navara & Nelson, 2007). This natural light-dark cycle is disrupted in the presence of artificial light: night-time lighting levels in cities can reach 100 lx (in contrast, a full moon on a cloudless night in a dark area will generate approximately 0.1 lx of illuminance). The presence of artificial light at night is increasingly a global environmental problem, affecting 23% of the world's nonpolar land surfaces, including 88% of Europe and 50% of the U.S.A. (Falchi et al., 2016). Mounting evidence suggests that artificial light at night influences biological processes and probably reduces the reproductive fitness of organisms living in its presence (Gaston,

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Visser, & Holker, 2015). Specifically, artificial light has been shown to substantially affect the reproductive behaviours of nocturnal and crepuscular species in urban areas. Compared to their rural conspecifics, urban European songbirds (including great tits, *Parus major*, robins, *Erithacus rubecula*, and the European blackbird, *Turdus merula*) commence their dawn chorus earlier (Da Silva, Samplonius, Schlicht, Valcu, & Kempenaers, 2014; Kempenaers, Borgström, Loës, Schlicht, & Valcu, 2010). Similarly, male green frogs, *Lithobates clamitans*, inhabiting ponds illuminated by artificial light invest less time calling for mates than frogs inhabiting naturally dark ponds (Baker & Richardson, 2006). Male winter moths, *Operophtera brumata*, located in areas affected by artificial light are less receptive to female pheromones (Van Geffen et al., 2015b) and experimental laboratory data suggest this behavioural shift is probably related to light-induced chemical disruption to the female sex pheromones (Van Geffen et al., 2015a). Despite increasing support for the impact of artificial light at night on reproductive behaviours, few studies have compared the effect of lifetime exposure to variation in light intensity on both mating behaviour and courtship communication (sexual signalling) simultaneously.

Here we determined whether lifetime exposure to four varying ecologically relevant intensities of artificial light at night affect the mating and courtship behaviour and acoustic sexual signalling of a common insect, the Australian black field cricket, *Teleogryllus commodus* (Orthoptera: Gryllidae). This species is an ideal model with which to examine the effects of artificial light on reproductive behaviours. It is both crepuscular and nocturnal, and ubiquitous in both urban and rural habitats which are varyingly affected by different intensities of artificial night lighting (Robinson, 2005). In addition, male *T. commodus* produce courtship calls that attract females acoustically, and these songs are strongly sexually selected (Hall, Bussière, Hunt, & Brooks, 2008).

METHODS

Experimental Animals

Teleogryllus commodus were sourced from a stock population that originated from rural Kinglake, Victoria, Australia (latitude −37.463959, longitude 145.198059). Crickets were maintained in the laboratory (26 °C, 12 h dark (0 lx illuminance): 12 h light (500 lx) photoperiod) for 10 generations prior to this experiment. This allowed us to minimize possible historical environmental and genetic effects to isolate the singular role of chronic exposure to artificial light at night on animals that have been exposed to only dark night for generations. Experimental adults were from one of 14 families that had been individually reared from the egg stage under standard rearing conditions (Durrant et al., 2015), in one of four different light at night treatments (0 lx = 22 females, 25 males, 1 lx = 22 females, 30 males, 10 lx = 25 females, 27 males, 100 lx = 32 females, 30 males; see full description of lighting treatments below). Crickets were weighed prior to all experiments, and hind femur lengths measured after either their natural death or following euthanasia by freezing at the completion of experiments. The average femur length was used as a proxy of body size (Durrant et al., 2015; Mousseau & Roff, 1989); body condition was subsequently determined from the residuals of a regression analysis of femur length and body weight (Danielson-François, Fetterer, & Smallwood, 2002; Gray & Eckhardt, 2001).

Ethical Note

As *T. commodus* is a common insect species not protected by conservation laws in Australia, no permits or ethics approval was needed to collect, house or utilize them in the laboratory. We housed adult crickets individually in our study to reduce the chances of injury and stress due to intraspecific aggression, and provided ample food, water and shelter throughout the experiment. Disturbance and unnecessary handling were kept to a minimum. Crickets were humanely euthanized by freezing (recommended method for ectotherms) at the conclusion of experiments (average age = 33 days posthatching), as they were nearing the end of their natural life cycle (crickets in the laboratory typically live 40–50 days posthatching; Durrant, Botha, Green, & Jones, 2017).

Light Treatments

We created different artificial night lighting environments using four purpose-built incubators (retrofitted Westinghouse: model number WRM4300WB-R; LED Teknik LT 2102B lighting) which each had identical daytime lighting (2600 lx, 6800 K), temperature (28 °C) and light cycle (12:12 h day:night) but varied in their nighttime lighting (all at 5900 K): 0 lx (control: complete night darkness), 1 lx (10 times full moonlight on a cloudless night), 10 lx (average light scattered from urban street lighting) and 100 lx (bright urban lighting). To ensure no incubator bias, light treatments were rotated between incubators thrice weekly and experimental individuals were rotated within the incubators every 2 days. Newly moulted (day 0) adults were sexed and transferred to individual containers (15 × 9 cm and 5 cm high) containing a piece of egg carton for shelter, and ad libitum water and food (three dry cat food pellets; Friskies Senior, Rhodes, NSW, Australia). Adults were maintained in this manner for 21 (±3) days; food and water were changed every 2–3 days.

Courtship and Mating Behaviour

At 21 (±3) days after final moult, each experimental adult cricket was weighed (to the nearest 0.01 mg) and then paired with a virgin stock cricket of the opposite sex (reared under standard conditions; see Durrant et al., 2015) of similar age. This age was chosen to ensure that all crickets were sexually mature and in peak breeding condition (the peak mating period for laboratory crickets begins at approximately 14–21 days after final moult; Durrant et al., 2017). Mating trials were no-choice trials, and the stock mate provided a comparable backdrop against which the four light treatments could be equitably compared. For each trial, the experimental individual was transferred to a plastic mating arena (15 × 9 cm and 5 cm high) and a stock cricket added within a minute. Pairs were observed for 45 min or until we observed the transfer of a spermatophore from the male to the female genital (defined hereafter as a successful mating, following Gress & Kelly, 2011 and Worthington, Gress, Neyer, & Kelly, 2013). If the pair did not mate within 45 min, the stock individual (male or female depending on the experimental sex being tested) was replaced and the procedure repeated. If this second mating trial was unsuccessful, the experimental individual was considered unmated. For all trials, we recorded the number of partners (1 or 2), the time to first male courtship call (s), the number of female mountings lasting at least 1 s (in crickets, females mount males prior to copulation but they may also leave the male prior to copulation), latency to successfully mate (s), duration of mating (s) and the overall mating success (yes/no). Mating trials commenced approximately 3 h before laboratory sunset (which is when crickets typically begin calling in the laboratory and field).

Bioacoustic Analyses

One week after each cricket's mating trial (adult age = 28 ± 3 days), a subset of 84 experimental male crickets (0 lx = 18 males, 1