

## 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.

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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 varying 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 ( $26^{\circ}\text{C}$ , 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 ( $28^{\circ}\text{C}$ ) 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 ( $15 \times 9$  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 ( $\pm 3$ ) days; food and water were changed every 2–3 days.

### Courtship and Mating Behaviour

At 21 ( $\pm 3$ ) 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 ( $15 \times 9$  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 =  $28 \pm 3$  days), a subset of 84 experimental male crickets (0 lx = 18 males, 1

lx = 21 males, 10 lx = 21 males and 100 lx = 24 males) were assayed to assess whether variation in the intensity of artificial light at night affected male courtship song. As for the mating trials, males were weighed prior to the commencement of the assay. Paired comparisons revealed that the subset of males contributing to the acoustic assays were comparable in key parameters to the remaining males (paired *t* tests: mean body size:  $P = 0.23$ ; body condition:  $P = 0.56$ ; time to first call:  $P = 0.869$ ; mating success in previous assay (above):  $P = 0.315$ ; number of female mountings:  $P = 0.412$ ), and can thus be deemed a representative subset of males from the mating trials.

#### Audio recordings

Acoustic assays were run in a sound-controlled environment (28 °C, 350 lx) acoustically removed from all other crickets. As in the mating trials, acoustic assays commenced approximately 3 h before laboratory sunset. Each trial was conducted in a large foam-lined (5 cm thick acoustic foam covering five sides) plastic acoustic chamber (30 × 24.5 cm and 12.5 cm high), which housed a smaller plastic courtship arena (15 × 9 cm and 5 cm high). At the start of a trial, the experimental male was placed in the arena and left for 2 min prior to the introduction of a randomly selected and weighed stock female (reared under standard conditions and control photoperiod; see Durrant et al., 2015), as in the courtship and mating behaviour assays (above). The pair was left for a further 2 min to acclimatize before recording commenced. After this time, we commenced recording once a male produced his first courtship call, and ceased recording after a further 10 min (regardless of the number of calls made during this period). If the male failed to call within 20 min, the trial was stopped, the stock female replaced and the process repeated. Males that failed to commence calling with the second female were discarded from further analysis (number of discarded males in 0 lx = 1, 1 lx = 2, 10 lx = 0, 100 lx = 3). Recordings were made using a Yoga EM-2.1 omnidirectional tie-clip condenser microphone, mounted inside the centre of the lid of the acoustic arena, connected to a Digitech XC-0383 digital voice recorder.

#### Bioacoustic analyses

Bioacoustic analyses of courtship calls were conducted using version 2.1.1 of Audacity audio recording and editing software (Audacity Team, 2015) and were completed by a single investigator (G.R.H.) who was blind to cricket ID and their associated light treatment. The order in which recordings were analysed was randomized using a random number generator. Each recording was first filtered to exclude ambient noise <3.5 kHz and >6.0 kHz (Simmons, Thomas, Simmons, & Zuk, 2013). For each 10 min recording, the number of courtship or aggressive calls was visually identified (Gerhardt & Huber, 2002) and counted. The number of aggressive songs sung by males was very low on average (mean ± SE across light treatments =  $1.47 \pm 0.35$ ; range 0–15) and was excluded from statistical analyses as a result. The total length of all courtship calls sung during the 10 min recording period was summed to assess the amount of time spent courtship calling. To assess variation in courtship call parameters, five calls were selected at random for detailed analysis by numbering all calls made by males during the period and analysing five chosen by a random number generator (Hall et al., 2008). Where less than five courtship songs were recorded for a given male all calls were analysed ( $N = 1$  in 0 lx [3 calls], 2 in 1 lx [1 and 3 calls], 3 in 10 lx [1, 1 and 4 calls] and 1 in 100 lx [3 calls]). The courtship call of *Teleogryllus* species is composed of a single chirp followed by a variable number of trills, each with multiple pulses (Fig. 1). We measured several parameters (Table 1) in the chirp and trill elements that are considered important for sexual selection in this genus (Hall et al.,

2008; Rebar, Bailey, & Zuk, 2009; Simmons et al., 2013). Pulse characteristics (e.g. chirp pulse duration or trill pulse interval, see Table 1) were measured on the first, fifth and 10th pulses of the chirp or first trill, and averaged to derive a mean value for that song. All measured elements were then averaged across the analysed songs for that individual to derive a single mean value for each element (Hall et al., 2008).

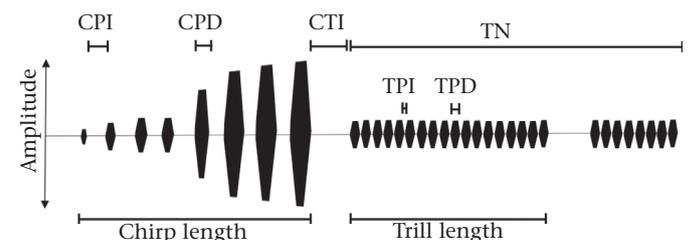
#### Statistical Analyses

##### Mating behaviour

Exploration and analyses of mating data were performed in JMP (Version 12, SAS, Cary, NC, U.S.A.). Variables were assessed for normality prior to analysis. Variation in the probability of mating was assessed using nominal logistic models; courtship and mating behaviours were determined using standard least-squares regression models. Maximal models included light treatment (0 lx, 1 lx, 10 lx 100 lx), sex, partner number (1, 2) and all interactions. Femur length, partner weight, days to adult and minutes prior to night were also added where appropriate, and family was included as a random effect. The interaction of body condition and light treatment was also examined to determine whether light treatment had indirect effects on mating and courtship behaviour by affecting body condition. Number of female mountings was analysed using mating success as a fixed effect, in addition to the terms described above. Each model was reduced using hierarchical removal of all terms with a significance of  $P > 0.1$  (except the designated light treatment). Significance testing was completed using type III tests, and unless otherwise stated, significant factors were assessed using post hoc Tukey's *t* tests.

##### Bioacoustic recordings

The number of courtship calls sung by males and the total time spent calling were each square-root transformed to meet normality assumptions before being tested in generalized linear mixed-effects models (GLMM) with light treatment, male average femur length, the interaction of male body condition with light treatment, female partner weight and recording temperature as fixed effect predictors, and previous mating success (in mating behavioural trials) and family as random effects, using the R (R Core Team, 2016) packages lme4 (Bates, Maechler, Bolker, & Walkder, 2015) and lmerTest (Kuznetsova, Brockhoff, & Christensen, 2016). Each model was reduced using hierarchical removal of all fixed effect terms with a significance of  $P > 0.1$  (except the designated light treatment). All significance testing was done using type III tests, and significant factors were assessed using post hoc Tukey's *t* tests. Individual call components were checked for normality and homogeneity of variance, and transformed as necessary (Table 1). Trill pulse number (TPN) and chirp pulse number (CPN) were highly correlated with trill length ( $r = 0.98$ ) and chirp length ( $r = 0.88$ ), respectively, and so were removed from the analysis. We



**Figure 1.** A waveform diagram showing a stylized courtship call of *Teleogryllus commodus*, with various analysed elements of the chirp and trill annotated (see Table 1 for abbreviations and details).

**Table 1**  
Elements of the courtship song analysed, along with their abbreviation used in this study

Song element	Abbreviation	Description	Transformation
Song length	–	Total amount of time (s) for a complete song (chirp+trills)	Transformed $\sim -0.25$
Chirp length	–	Total amount of time (s) from the start to the end pulse of the chirp	Inverse
Trill length	–	Total amount of time (s) of the first trill in the song	Square-root
Chirp frequency	CF	Dominant frequency (Hz) of the chirp element	–
Trill frequency	TF	Dominant frequency (Hz) of the first trill element	–
Chirp pulse number	CPN	The total number of pulses in the chirp element	ln
Chirp pulse rate	CPR	The total number of pulses in the chirp (CPN) divided by the total chirp length	–
Chirp pulse duration	CPD	Mean duration (s) of the first, fifth and 10th pulses of the chirp	Inverse
Chirp pulse interval	CPI	Mean duration (s) of the interval between pulses 1–2, 5–6 and 10–11 of the chirp	Inverse
Chirp – trill interval	CTI	The duration (s) of the interval between the end of the last pulse of the chirp and the beginning of the first pulse of the first trill	$\sim -0.4$
Trill number	TN	The total number of trill elements in the song	Square-root
Trill pulse number	TPN	The total number of pulses in the first trill element	Square-root
Trill pulse rate	TPR	The total number of pulses in the chirp (TPN) divided by the trill length	$\sim -0.5$
Trill pulse duration	TPD	Mean duration (s) of the first, fifth and 10th pulses of the first trill	Square-root
Trill pulse interval	TPI	Mean duration (s) of the interval between pulses 1–2, 5–6 and 10–11 of the first trill	Inverse

Many of these elements are illustrated in Fig. 1.

standardized all remaining courtship call elements to mean = 0, SD = 1 prior to analysis, as recommended by Drayton, Hall, Hunt, and Jennions (2012) and Hall et al. (2008). To determine whether there was an overall structural difference between courtship calls of males in the different light treatments, we conducted a linear discriminant analysis of all measured courtship call elements (as in Rebar et al., 2009) using the R MASS package (Venables & Ripley, 2002), and assessed the significance of overall structural differences between light treatments using MANOVA.

## RESULTS

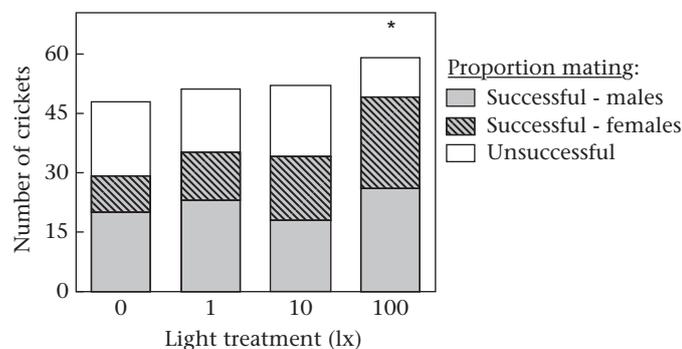
### Mating Behaviour

#### Probability of a successful mating

Overall, there was a significant effect of light treatment on the probability of mating (logistic regression:  $\chi^2_3 = 8.18$ ,  $N = 320$ ,  $P = 0.042$ ): 100 lx animals were more likely to mate than 0, 1 or 10 lx individuals (Fig. 2). Experimental males were more likely to mate with a stock female than experimental females were to mate with a stock male (significant effect of sex:  $\chi^2_1 = 11.78$ ,  $P = 0.0006$ ). There was no significant interaction between light treatment and sex ( $P > 0.10$ ).

#### Courtship and mating behaviour

The time to the first call (ln-transformed data) was comparable across light treatments (Table 2, Fig. 3a) but was shorter for the first



**Figure 2.** Proportion of *T. commodus* crickets mating successfully after lifetime exposure to various intensities of artificial light at night. \* $P < 0.05$ , post hoc multiple comparisons with all other treatments.

than the second partner (significant effect of partner: Table 2, Fig. 3a; mean  $\pm$  SE time to first call of Partner 1 =  $368.32 \pm 38.33$  s; Partner 2 =  $627.24 \pm 81.31$  s) and varied with body condition across light treatments (significant interaction between light treatment and body condition: Table 2). Post hoc analyses (controlling for the partner number but separated by light treatment) revealed a negative relationship between body condition and time to first call for 0 lx ( $P = 0.001$ ;  $\beta \pm$  SE =  $-0.61 \pm 1.74$ ) and 100 lx individuals ( $P = 0.0005$ ;  $\beta \pm$  SE =  $-3.77 \pm 1.28$ ) but no significant pattern for 1 lx and 10 lx individuals (both  $P > 0.6$ ).

Latency to mate (Table 2, Fig. 3b) was comparable across the four light treatments (Table 2), but was higher in the second compared to first trials (significant effect of partner number: Table 2). The significant interaction between partner number and sex revealed that this was driven largely by differences in males rather than females across partners (significant interaction between partner number and sex: Table 2, Fig. 3b). There were no main effects of either light treatment or partner number on mating duration (Table 2); however, there was a weak interaction between light treatment and partner number (Table 2, Fig. 3c; mating duration decreased for the second partner for 10 lx animals but increased for the second partner in all other light treatments).

The number of times a female mounted a male (Table 2) was unrelated to the main effect of either light treatment or sex; however, there was a significant interaction between these two variables (Table 2, Fig. 4). Post hoc comparisons within each sex revealed that 100 lx and 10 lx females mounted males significantly less often than 0 or 1 lx females (planned contrasts:  $P < 0.05$ ; Fig. 4a). In contrast, 100 lx males were mounted significantly more frequently than either 0 or 1 lx males (planned contrasts:  $P < 0.05$ ; Fig. 4b). The number of mounts was higher in trials that resulted in a successful mating than in trials that resulted in no mating (significant effect of successful mating; Table 2).

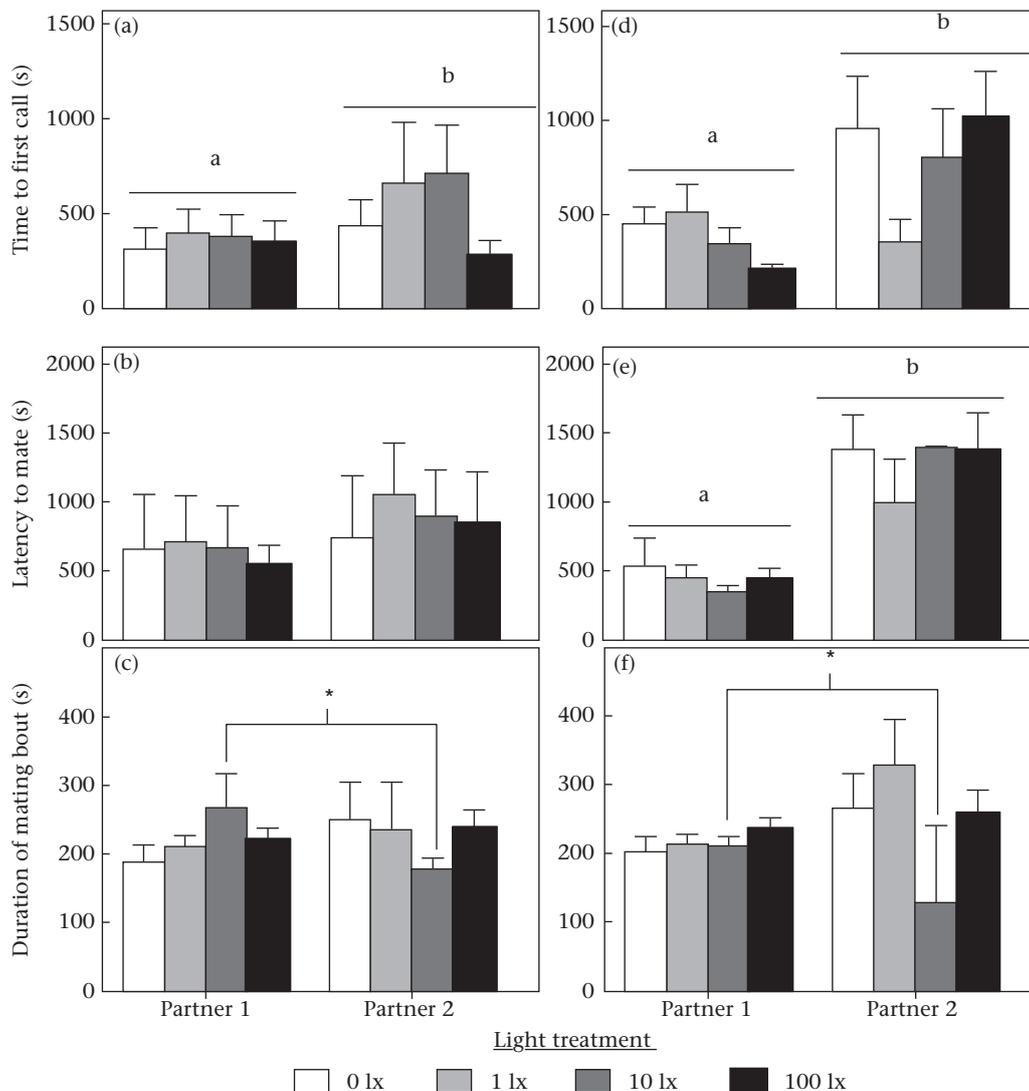
### Bioacoustic Analyses

#### Number of courtship songs and time spent calling

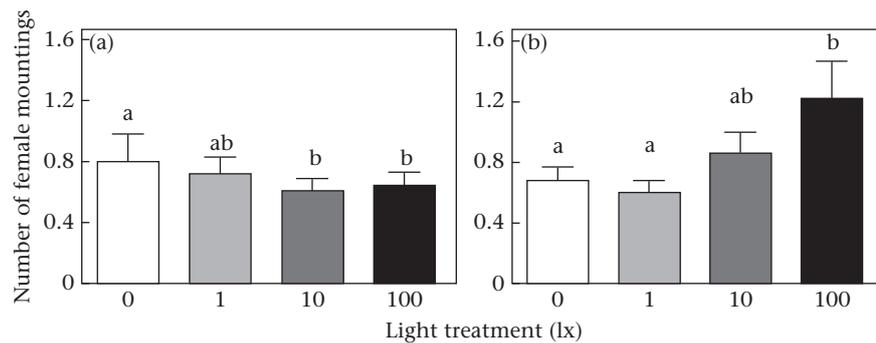
The number of courtship calls sung by male crickets was comparable across the four light treatments (GLMM:  $F_{3,51} = 1.53$ ,  $P = 0.22$ ; mean  $\pm$  SE number of calls sung across light treatments =  $36.97 \pm 3.53$ ; range 0–101). There was a significant positive relationship of number of courtship calls with male body condition (GLMM:  $F_{1,48} = 5.66$ ,  $P = 0.021$ ;  $\beta \pm$  SE =  $3.01 \pm 1.26$ ). While it varied only slightly across trials (mean  $\pm$  SD =  $28.16 \pm 0.46$  °C; range 2.65 °C), temperature also positively

**Table 2**  
Effects of light treatment and other significant variables on mating behaviours of *T. commodus* exposed to artificial light at night

Model	Means±SE for light treatments				Statistic	P
	0 lx	1 lx	10 lx	100 lx		
Time to first call (s, ln transformed)						
Light treatment	472.45±70.49	485.75±89.03	482.54±79.39	351.29±57.53	$F_{3,200}=2.08$	0.10
Body condition					$F_{1,200}=7.92$	0.005
Light treatment*Body condition					$F_{3,200}=3.80$	0.01
Partner (1, 2)					$F_{1,200}=11.90$	0.0007
Latency to mate (s)						
Light treatment	835.70±54.02	714.89±119.96	596.91±109.78	671.75±97.28	$F_{3,138}=0.12$	0.95
Partner (1,2)					$F_{1,138}=23.30$	<0.0001
Sex					$F_{1,138}=0.48$	0.49
Partner*Sex					$F_{1,138}=5.32$	0.02
Mating duration (s)						
Light treatment	224.66±18.26	241.42±17.46	210.79±15.01	232.98±10.79	$F_{3,136}=1.90$	0.13
Partner (1,2)					$F_{1,136}=2.20$	0.14
Light treatment*Partner					$F_{1,136}=3.32$	0.02
Female mounting attempts						
Light treatment	0.74±0.10	0.65±0.07	0.73±0.08	0.90±0.13	$F_{3,306}=0.68$	0.57
Sex					$F_{1,306}=0.01$	0.94
Light treatment*Sex					$F_{3,306}=4.58$	0.004
Successful mating? (0,1)					$F_{1,306}=39.13$	<0.0001



**Figure 3.** Courtship and mating behaviours of *T. commodus* crickets reared under different night-time lighting intensities (0, 1, 10 and 100 lx) in relation to partner number and sex. (a, b, c) females, (d, e, f) males. (a, d) Time to first call, (b, e) latency to mate and (c, f) duration of mating bout. Bars represent means ± SE. Different lowercase letters indicate significant effects of partner number. An asterisk indicates significant interactions of light treatment and partner. See Table 2 for full statistical details.



**Figure 4.** Mean  $\pm$  SE number of female mountings across mating trials of *T. commodus* crickets exposed to artificial light at night throughout development. (a) Mountings by females and (b) mountings received by males. Bars represent means  $\pm$  SE. Different lowercase letters indicate significant differences between treatments (planned contrasts within each sex).

affected the number of courtship calls (GLMM:  $F_{1,50} = 5.52$ ,  $P = 0.023$ ;  $\beta \pm SE = 2.32 \pm 0.99$ ). There was no effect of light treatment on the total time spent courtship calling (GLMM:  $F_{3,51} = 1.41$ ,  $P = 0.25$ ; mean  $\pm$  SE time spent calling across light treatments =  $178.46 \pm 17.61$  s; range 0–589.83 s), but a significant positive relationship between male body condition and time spent calling (GLMM:  $F_{1,51} = 5.59$ ,  $P = 0.02$ ;  $\beta \pm SE = 6.71 \pm 2.84$ ). Recording temperature was also positively related to the time spent calling (GLMM:  $F_{1,51} = 6.33$ ,  $P = 0.02$ ;  $\beta \pm SE = 5.62 \pm 2.23$ ).

#### Courtship call structure

Overall courtship call structure did not vary significantly between light treatments (MANOVA: Wilks = 0.464, approximate  $F_{39,161} = 1.22$ ,  $P = 0.20$ ), and discriminant analysis failed to accurately discriminate between light treatment groups based on multivariate call structure (overall LDA accuracy = 30%, 0 lx accuracy = 43.75%, 1 lx = 21.05%, 10 lx = 18.75%, 100 lx = 36.84%).

## DISCUSSION

Our study found that chronic lifetime exposure to the highest level of artificial light at night (100 lx; the equivalent of a brightly lit urban area) increased the probability of a successful mating, and potentially disrupted precopulatory mating behaviour (mountings) in *T. commodus*. These effects were not observed at lower light levels, and were often sex specific. Uniquely, we also demonstrated that these behavioural effects were independent of acoustic sexual signalling, as courtship calling was largely robust to light treatments, with no effects on call effort or structure. Combined, our results suggest that although our laboratory population of *T. commodus* may be somewhat resilient to low levels of artificial light, the presence of chronic exposure to high-intensity night lighting may have sex-specific effects on aspects of reproductive behaviour in this species that warrant further study.

Chronic exposure to bright light at night (10–100 lx) altered specific aspects of mating behaviour in *T. commodus*, affecting the rate of precopulatory mountings and overall mating success. Specifically, experimental females reared under 10 and 100 lx at night engaged in less mounting when provided with a stock male, while experimental males reared under 100 lx were mounted more often prior to a successful mating. We note that mating trials in our study were stopped when a successful mating occurred through the transfer of a spermatophore. Thus, an increase in the number of mountings within a trial is indicative of an increase in mating discrimination by a female (it is the female that mounts the male and determines in part whether a spermatophore is transferred successfully) and/or decreased mating efficiency by a male (he initiates spermatophore transfer). For females, therefore, it appears

that the presence of higher levels of artificial light at night during rearing may reduce mate discrimination (they typically mated immediately following their first mounting); in contrast, males reared under 100 lx were discriminated against (they were mounted and rejected more often than in other treatments). This shift in mate choice may have important fitness consequences. Our experimental design meant that females remained with a male for 45 min regardless of their initial preference, and this may have resulted in an inflated mating success for 100 lx males (and could explain the positive relationship between number of mountings and mating success). A more realistic design, where a female could leave a male entirely rather than remount the same male might have yielded very different results. Similarly, introducing a second mate if the first was unsuccessful may have also artificially inflated mating success rates in our study, and masked the true consequences of initial failure to mate in the wild. While a lack of mating efficiency may not be a deficit in the laboratory, in nature this may have an additional fitness cost, as animals are particularly vulnerable to predation during courtship and mating (Endler, 1987; Magnhagen, 1991). This risk may be amplified in artificially lit environments (Endler, 1987) where several insectivorous predators, for example multiple species of anurans and lizards (reviewed by Perry, Buchanan, Fisher, Salmon, & Wise, 2008), spiders (Heiling, 1999) and bats (Minnaar, Boyles, Minnaar, Sole, & McKechnie, 2014; Rydell, 1992), have been shown to congregate and increase their feeding intake. Whether these trade-offs exist in the wild remains to be determined.

In contrast to the behavioural results of crickets exposed to high-intensity lighting, we found that chronic exposure to artificial light at night, at any intensity, did not affect acoustic sexual signalling in this species, with no changes to calling effort or overall multivariate structure. This may explain the relative willingness of female crickets from all light treatments to mount males initially, as the courtship call is largely used by males to initiate female mounting (Alexander, 1961). It also means that the effects on mating behaviours described above were decoupled from acoustic sexual signalling. Given the importance of these signals for mating and courtship behaviour of *Teleogryllus* species (Hall et al., 2008; Hill, 1974; Hill, Loftus-Hills, & Gartside, 1972; Simmons et al., 2013; Tregenza, Simmons, Wedell, & Zuk, 2006), this is an interesting result. While female crickets may still have been attracted to the courtship song of all treatment males, the increased number of mounting attempts on 100 lx males prior to mating suggests a reduced efficiency and/or increased discrimination of females in mating with these individuals. The underlying mechanism generating these differences in female mounting behaviour with respect to artificial light is unknown; however, it is possible that males vary in their cuticular hydrocarbon (CHC) profiles, close-range cues used

by females to assess males after calling has taken place (Alexander, 1961). While we did not examine possible CHC differentiation in our study, CHC chemical profiles are strongly sexually selected in the closely related cricket species, *Telegryllus oceanicus* (Simmons et al., 2013; Thomas & Simmons, 2009), and have the capacity to be altered due to environmental changes in selection pressures (Simmons, Thomas, Gray, & Zuk, 2014). We also did not examine long-distance attraction calls in our study, as our interests lay in the more immediate effects of artificial night lighting on courtship and mating behaviour once mates were in close contact. However, as female crickets in the wild will travel across open terrain to locate males based on their attraction calls (Hill, 1974), and thus potentially expose themselves to artificial light as a result, a future analysis of possible differentiation of attraction calls of wild crickets from varying light environments may prove relevant. The differentiation of courtship signals in general has important implications for population differentiation and speciation (Panhuis, Butlin, Zuk, & Tregenza, 2001), and so the possible differentiation of these signals in urban, brightly lit environments may have broader impacts for this species that remain to be investigated.

The effects of chronic exposure to artificial light at night on mating and courtship behaviours seen in this study were observed only in the brightest treatments, and were not seen at the lowest lux level. It is possible that our laboratory population, and benign laboratory conditions in particular, may have selected for tolerance or mitigated against effects of artificial light at these lower levels of brightness. For example, ad libitum food and water could have masked the consequences of a potentially stressful light environment on reproduction by driving compensatory behavioural shifts such as foraging not examined in this study (Buchanan, Spencer, Goldsmith, & Catchpole, 2003). In addition, our experimental adults were reared from embryos in varying night-time lighting conditions which may have resulted in strong selection at the egg and juvenile stages, leaving only individuals that were more tolerant of artificial light surviving to the adult stage. Finally, for those individuals that survived to adulthood and participated in the present study, as discussed above, the experimental design of our mating trials may have also mitigated against large effects. For these, and other reasons, it is difficult to make definitive inferences on the effects of artificial light at night on wild crickets based purely on the results of our laboratory study. However, studying the effects of artificial night lighting in this context does allow us to carefully examine a singular stressor that is extremely challenging to do in the wild, where numerous other confounding variables are also at play (Gaston, Duffy, Gaston, Bennie, & Davies, 2014). It may be, therefore, that our results represent a minimum level of impact of artificial light on the mating and courtship behaviours of crickets, and we might expect the impacts of this stressor to be even greater on wild populations, outside of the confines of a relatively benign laboratory. On the other hand, our study may also hint at a potential robustness of *T. commodus* mating and courtship behaviour to the levels of artificial light at night seen in many urban habitats (i.e. <100 lx). Being both crepuscular and nocturnal, this species does already naturally cross the diurnal–nocturnal boundary in its activity, and is found in a wide variety of urban and peri-urban habitats (Robinson, 2005), where males have been observed calling in artificially lit city streets (Hill, 1974; G.R. Hopkins, personal observation). Examinations of wild crickets, across a variety of night lighting environments, is a logical next step in our investigations.

This study examined the potential impacts of artificial light at night on mating and courtship behaviour and acoustic sexual signalling in a common nocturnal and crepuscular insect. While we found effects of artificial light on mating and courtship behaviour only at the highest levels of lighting examined, and under chronic

conditions, in this species, it should be emphasized that for other species and/or other endpoints measured, the effects of artificial light at night may be revealed at much lower intensities or more acute durations than those in the present study, or may be dependent on spectral composition (Davies, Bennie, Inger, Hempel de Ibarra, & Gaston, 2013; Gaston et al., 2015; de Jong et al., 2016). The effects of chronic exposure to differing intensities of light at night on the reproductive behaviours of most animals have yet to be studied. As artificial light at night continues to spread, and the world's dark areas become brighter (Falchi et al., 2016), chronic exposure to bright night-time conditions are likely to become the norm for many urban animals. More detailed work needs to be completed across taxa to fully understand the biological and ecological ramifications of this pervasive anthropogenic stressor (Gaston et al., 2015).

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