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Artificial light at night alters the sexual behaviour and fertilisation success of the common toad

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Abstract

Artificial Light At Night (ALAN) is an emerging pollution, that dramatically keeps on increasing worldwide due to urbanisation and transport infrastructure development. In 2016, it nearly affected 23% of the Earth's surface. To date, all terrestrial and aquatic ecosystems have been affected. The disruption of natural light cycles due to ALAN is particularly expected for nocturnal species, which require dark periods to forage, move, and reproduce. Despite the growing number of studies on this subject, our knowledge on the direct influence of nocturnal lighting on amphibians, the most endangered vertebrate, is still scarce. Apart from chiropterans, amphibians contain the largest proportion of nocturnal species among vertebrates exhibiting an unfavourable conservation status in most parts of the world and living in ALAN polluted areas. To better understand the consequences of ALAN on the breeding component of amphibian fitness, we experimentally exposed male breeding common toads (*Bufo bufo*) to ecologically relevant light intensities of 0.01 (control), 0.1 or 5 lux for 12 days. At mating, exposed males took longer than controls to form an amplexus, *i.e.*, to pair with a female, and broke amplexus before egg laying, while controls never did. These behavioural changes were associated with fitness alteration. The fertilization rate of 5 lux-exposed males was reduced by 25%. Salivary testosterone, which is usually correlated with reproductive behaviours, was not altered by ALAN. Our study demonstrates that ALAN can affect the breeding behaviour of anuran species and reduce one component of their fitness. Given the growing importance of ALAN, more work is needed to understand its long-term consequences on the behaviour and physiology of individuals. It appears essential to identify deleterious effects for animal populations and propose appropriate management solutions in an increasingly brighter world.

Capsule summarizing the main finding: Artificial light at night decreased the capacity of male *Bufo bufo* to form a pair with a female and reduced their fertilization success.

46 **Key words:** Fitness, reproduction, amphibian, anthropogenic light pollution, behaviour

Introduction

In recent decades, the rapid growth in the world's population has led to a sharp increase in human activities necessary to support this growth. One consequence of the expansion of urban areas along with the development of transport infrastructures (Grimm et al. 2008; Gaston et al. 2013) is the major increase of light levels at night. In 2016, nearly 23% of the Earth's surface, 88% of Europe and almost half of the United States experienced brightness levels higher than light levels at night in natural ecosystems (Falchi et al. 2016). Artificial Light At Night (ALAN) dramatically expanded. From 2012 to 2016, Earth's artificially lit outdoor area grew by 2.2% per year, with a total radiance increase of 1.8% per year (Kyba et al. 2017). One of the major effects of ALAN is the disruption of the natural photoperiod, which is one of the most important cues for biological timing (Bradshaw & Holzapfel 2010). Among all organisms, nocturnal species, which represent a large proportion of biodiversity, 28% of vertebrates and more than 60% of invertebrates (Hölker et al. 2010), are most likely to experience and to be affected by ALAN (Buchanan 2006; Duffy et al. 2015; Desouhant et al. 2019). Nocturnal artificial light is known to affect a wide range of physiological and behavioural phenomena, such as migration (Van Doren et al. 2017), orientation (Tuxbury & Salmon 2005), activity (*e.g.* Le Tallec et al. 2013; Pulgar et al. 2019; Touzot et al. 2019), foraging (Czarnecka et al. 2019), energy balance (*e.g.* Welbers et al. 2017; Touzot et al. 2019) and hormonal synthesis (*e.g.* Brüning et al. 2015; Newman et al. 2015). These effects on individuals have the potential to alter population dynamics (*e.g.* Gaston et al. 2014a; Grubisic et al. 2017; Sanders et al. 2018).

Despite the evidence of profound effects of ALAN on life history traits across different taxonomic groups with important ecological consequences (*e.g.* Gaston et al. 2014b; Knop et al. 2017; Bennie et al. 2018), we still have little information on the direct effects of ALAN on the fitness of individuals although its assessments are key elements in conservation.

Fitness, *i.e.* the average contribution to the gene pool of the next generation that is made by individuals of a specified genotype or phenotype in a given environment, is often estimated through measurements of mortality and breeding success. Most of the studies conducted to date focused on the effects of ALAN on the mortality component of fitness (*e.g.* Rodríguez et al. 2014; Willmott et al. 2018). The breeding component of fitness has been less examined, even if. However, observation-based studies did not control for other environmental factors that are often correlated with ALAN in the wild (temperature of urban environment, habitat fragmentation, prey availability...), while most experiment-based studies used higher intensities than those actually encountered by individuals in their habitat (May et al. 2019). a growing number of studies (reviewed in Ouyang et al. 2018) reported that the timing and the physiology of seasonal reproductive processes differ between individuals living in lit areas and their conspecifics living in darker areas. Alterations of the period of functional development of reproductive organs, reproductive hormonal synthesis (estradiol, ketotestosterone), number of eggs produced by females, egg hatchling success, number of offspring and birth schedule in response to ALAN exposure were found in mammals (*e.g.* Le Tallec et al. 2015; Robert et al. 2015), birds (*e.g.* Dominoni et al. 2013; de Jong et al. 2015), fishes (*e.g.* Brüning et al. 2018, Fobert et al. 2019) and insects (*e.g.* Willmott et al. 2018; McLay et al. 2018). Photoperiod modifications have also been shown to affect sexual maturation and sex steroids synthesis, especially in fish (*e.g.* Norberg et al. 2004). The suppression of the dark period triggered a decrease of spermatocytes in male Asian toads, *Bufo melanostictus*, (Biswas et al. 1978) and a severe reduction of sexual calls in male green frogs, *Rana clamitans melanota*, (Baker & Richardson 2006). On contrary, other studies have found no effect of ALAN on the time for copulation or between mating and laying egg, on the number of eggs or egg sacs laid and on sperm viability (*e.g.* Durrant et al. 2018; McLay et al. 2018; Willmott et al. 2018). A recent study investigating the influence of ALAN on the

fertilization success of fish, ultimately showed no effect (Fobert et al. 2019). In view of the consequences of ALAN on the breeding behaviour and physiology, it is necessary to investigate the direct influence of ALAN on the breeding component of fitness in amphibians.

In this context, we experimentally studied the effect of three ecologically relevant light intensities at night (0.01, 0.1 or 5 lux), which correspond to light levels measured in areas hosting amphibians (Secondi et al. 2017), on both the reproductive behaviour and the fertilisation success of male common toads, *Bufo bufo*. This nocturnal amphibian is one of the most common and ubiquitous amphibians in France, and as a result is often a useful indicator of ecosystem health and function (Hilty & Merenlender 2000). Moreover, the common toad is an explosive breeding species with a breeding period lasting only a few days, thus limiting the number of pairing opportunities (Wells 1977). The operational sex ratio of this species is biased towards males, leading to scrambling competition among males to mate with a female. During the breeding season, common toads are frequently found in urban and peri-urban areas with wetlands (Beebee 1979), especially small ones (ponds for instances) which are subjected to ALAN (Secondi et al. 2017). In addition, due to their high nocturnal visual sensitivity, amphibian activities, such as foraging and breeding, are expected to be affected by changes in night brightness (Buchanan 2006; Grant et al. 2009; Yovanovich et al. 2017). We have previously shown that ALAN exposure decreased common toad activity during the night and increased allocation of energy to maintenance (Touzot et al. 2019). This suggested that breeding activities occurring at dusk or at night may be influenced by ALAN. Considering this, we predicted that (i) ALAN exposure would alter male breeding behaviour, particularly their ability to maintain pairing with a female, which is a costly activity for this species (Lengagne et al. 2007), (ii) these behavioural alterations could be due to changes in male testosterone concentrations, and (iii) ALAN exposure would reduce male fertilisation success.

Methods

122 *Animal collection and housing conditions*

123 A total of 60 male common toads were collected during the breeding season (8-9 March 2018)
124 in La Burbanche, France (45°N, 5°E). This site was chosen for its low levels of ALAN
125 regardless of weather conditions and the lunar phase (≤ 0.01 lux). Upon arrival at the animal
126 care facility (EcoAquatron, University of Lyon), males were weighed (LAB 800-3000,
127 precision: 0.1 g, B3C pesage, Sérénité) and housed individually in boxes (47 x 36 x 25 cm)
128 containing a 15 cm section of PVC tubing (diameter 10 cm) for shelter and 4 cm of litter that
129 was moistened daily. Ambient temperature and relative humidity were kept during the whole
130 experiment at $15.9 \pm 0.6^{\circ}\text{C}$ and $55.2 \pm 2.9\%$, respectively. In these conditions, males were
131 active during the night. Toads were fed *ad libitum* with live domestic crickets, *Acheta*
132 *domesticus*, except during the mating trials. Sixty females of common toad collected at the
133 same breeding site were grouped by 5 in large boxes (60 x 42 x 39 cm) with 10 cm of litter
134 that was moistened daily. The ambient temperature was kept at $5.0 \pm 1.0^{\circ}\text{C}$. At this
135 temperature, females were inactive which prevented early spawning. Females were not
136 exposed to ALAN. Two days before the end of male ALAN exposure, the temperature of the
137 compartment containing females was increased to reach 15°C , a temperature which turns
138 them active during the night.

139 *Light treatments*

140 During 7-8 days of acclimation, toads were exposed during the daytime to 254 ± 4 lux (mean
141 \pm SEM) provided by light tubes (Philips Master TL-D 58W/865 and Exo Terra Repti Glo 2.0,
142 40W T8) and remained in the dark (< 0.01 lux) during the scotophase. After the acclimation
143 period, under the natural photoperiod at the date of the experiment, male toads were assigned
144 to one of the three treatments ($n=20$ for each treatment) and exposed to their respective light
145 treatment at night. To reproduce artificial light by night, we used white light-emitting diode

(LED) ribbons (white cold Light Plus, 6000-6500 K, 14W, 60 LED/m). White LEDs were chosen because they are increasingly used for street lighting worldwide (Falchi et al. 2016). A LED ribbon of 95 cm (57 LED) was fixed 20 cm above the bottom of each box for each light treatment (see Appendix Supplementary Material S1 for details). All the ribbons in a given light treatment were connected to a dimmer (manual dimmer, 12V max, 8A) and a laboratory power supply (15V/DC max, 3A), which allowed to finely adjust the light intensity of each light treatment. The boxes assigned to one light treatment were isolated from each other, so that the light intensities did not interfere with each other. Under the natural photoperiod at the date of the experiment, during the night, the control group was exposed to 0.01 lux, corresponding to the illuminance of a sky under clear conditions with a quarter moon (Gaston et al. 2013). The first experimental group was exposed to 0.10 lux (hereafter called the “0.1 lux-group”), corresponding to the illuminance of urban skyglow (Gaston et al. 2013). The second experimental group was exposed to 5 lux (hereafter called the “5 lux-group”), which corresponds to the light level of a residential street (Gaston et al. 2013). The daylight and 5 lux-group intensities were measured with a luxmeter (Illuminance meter T-10A, Konica Minolta). Intensities for the 0.10 lux and control groups were measured with a highly sensitive light meter (Sky Quality Meter-SQM-L, Unihedron). Consequently, to compare intensities, SQM measured values were converted into lux (see Appendix Supplementary Material S2 for details). Light intensities were measured at the bottom of the boxes and checked every week (0.01 ± 0.001 lux for the control group, 0.05 ± 0.01 lux for the 0.10 lux-group and 5.01 ± 0.06 lux for the 5 lux-group, mean \pm SEM). The exposure period lasted twelve days (D1 to D12), which corresponds to the average duration of a breeding period for this species in the wild (Wells 1977; Reading & Clarke 1983).

Reproductive behaviours

On D13, exposure to nocturnal light was stopped, and the litter contained in the box was replaced with 21 l of water (one-third of dechlorinated water and two-third of pond water) and tree branches for oviposition. Females that had not been exposed to ALAN were assigned to a partner (we have checked that both female body mass and the ratio between male and female size did not statistically differ between light treatments). During the following 27 hours, we recorded hourly males that amplexed a female. Amplexus can be either successful (*i.e.*, the male maintains the female until egg laying) or not (*i.e.*, the male stops maintaining the female in amplexus). As parameter we recorded the latency to obtain successful amplexus, *i.e.*, the duration required for males to form an amplexus leading to egg laying, and the separation rate, *i.e.*, the percentage of pairs that broke amplexus before egg laying. The following days (between 1 and 7 days), we determined the duration of amplexus before the clutch was laid. After laying, animals were removed, weighed and measured (Snout-to-Vent length, SVL) because previous studied showed that male pairing success was linked to their size (Davies & Halliday 1978; Lengagne et al. 2007) and fertilisation rate was linked to the ratio between male and female size (Davies & Halliday 1977). Four days after laying, we quantified the number of unfertilised eggs at the blastulation stage (Lengagne et al. 2007) and the total number of laid eggs. These data allowed us to calculate the fertilisation rate, *i.e.*, the proportion of fertilised eggs to the total number of laid eggs by a female.

Hormonal assay

On D13, males were weighed and measured, and a saliva sample was taken to measure their testosterone level. Sampling was carried out between 01:00 a.m. and 03:00 a.m., when testosterone concentration was the highest (see Appendix Supplementary Material S3 for details). Within the 2 min after capture, a dry cotton ball of known weight was introduced into the toad's mouth for 30 s to collect saliva. Each cotton ball was immediately weighed, placed into a microtube containing a filter to retain the cotton ball fibre during centrifugation and

stored at -80°C. We investigated testosterone levels in saliva, which is a non-invasive method commonly used for the detection of steroids in wild species, including amphibians (Kutsukake et al. 2009; Janin et al. 2012; Desprat et al. 2015). Saliva was extracted from the cotton ball by the addition of 500 µl of phosphate buffer (1 M phosphate solution containing 1% bovine serum albumin, 4 M sodium chloride, 10 mM ethylenediaminetetraacetic acid, and 0.1% sodium azide) and centrifugation (10 000 g at 4°C for 5 min). We used a testosterone Enzyme Linked ImmunoSorbent Assay (ELISA) kit (number 582701, Cayman Chemical Company, Ann Arbor, MI, U.S.A.), validated for amphibians (Desprat et al. 2015) and previously optimized for common toads, to analyze testosterone from saliva samples. Concentrations were calculated using a standard curve run on each plate and were estimated in 1 mg of saliva and expressed in pg mg⁻¹ of saliva.

Statistical Analysis

We performed a linear model (LM) to test the effects of the light treatment (0.01, 0.1, 5 lux) on male body mass gain during ALAN exposure and on male body mass on D13. To confirm that there were no confounding differences in body size between light treatments, we performed a LM to test the effect of light treatment (0.01, 0.1, 5 lux) on male SVL measurement and on the ratio between male and female size on D13. Successful amplexus latency data were analysed as survival data following a Weibull distribution. Light treatment (0.01, 0.1, 5 lux), male body mass, female body mass, male salivary testosterone concentrations, and the interactions between light treatment and male body mass and between light treatment and male salivary testosterone concentrations were included as explanatory variables. The effect of light treatment (0.01, 0.1, 5 lux), male body mass, female body mass, male salivary testosterone concentrations and the interactions between light treatment and male body mass and between light treatment and male salivary testosterone concentrations on the probability of separation before egg laying (separation = 1; no separation = 0) was

analysed through a generalized linear model (GLM) with a binomial distribution (logit link). As the results of the model indicated a marginal effect for the chosen significance threshold (5%), we calculated the effect size (Cohen's h for proportions). We performed a GLM with a Gamma distribution to test the effect of the light treatment (0.01, 0.1, 5 lux), male body mass, female body mass and the interaction between light treatment and male body mass on amplexus duration before egg laying. For this analysis, one value is missing in the 5 lux-group, as one female paired to a 5 lux-exposed male did not lay eggs. We performed a LM to test the effects of the light treatment (0.01, 0.1, 5 lux) and male body mass and their interaction on male salivary testosterone concentrations. For male salivary testosterone concentrations, one data point was excluded because the value was below the colour range of the ELISA kit. Male fertilisation rate was analysed through a GLM with a binomial distribution (logit link) using male body mass, female body mass, light treatment and the interaction between light treatment and male body mass as predictors. Because clutches are synchronous and, given the number of eggs to be counted, we counted fertilised eggs on a sub-sample of pairs ($n = 10$ for control, $n = 9$ for 0.1 lux and $n = 8$ for 5 lux), which corresponds to 94 102 eggs counted. For the analyses, the fitted models were simplified by a backward procedure to keep only the significant variables. For all models, multiple comparisons were performed using a Tukey adjusted post hoc test. All data were analysed using the software R 3.5.1 (R Core Team 2018) and the packages *lsmeans* (for successful amplexus latency and male fertilisation rate analysis), *pwr* (for the probability of separation before egg laying) and *survival* (for successful amplexus latency analysis).

Ethical note

The capture of common toads was authorized by the Préfecture de l'Ain (DDPP01-16-145) and by the French government (APAFIS#3655-2016011914372094) in accordance with the ethical committee of Lyon 1 University. The animal care structure "EcoAquatron" (University

of Lyon) received an agreement of veterinary services (approval DSV 692661201). At the end of the experiments, all the toads and the egg clutches were released to their original site.

Results

ALAN affected reproductive behaviours of males

(Fig. 1)

Male body mass gain during ALAN exposure was on average of 10.66 ± 1.27 g in the control group (mean \pm SEM) and did not significantly differ between the light treatments (see Appendix Supplementary Material S4 for details). At mating, on D13, male body mass, male SVL measurement and the ratio between male and female size did not significantly differed between the light treatments (see Appendix Supplementary Material S4 for details). For all light treatments, each male performed an amplexus with the female. However, ALAN strongly affected the reproductive behaviours of male common toads. First, successful amplexus latency significantly differed between pairs with an exposed male and pairs with a control male (Fig. 1; $X^2_2 = 14.01$, $P < 0.001$). Males exposed to 0.1 or 5 lux took much longer to achieve successful amplexus as shown by the 3-fold increase (Tukey post hoc test $P < 0.001$) and the 1.5-fold increase in latency (Tukey post hoc test $P = 0.004$), respectively, compared with control males. No significant difference was found between males exposed to 0.1 or 5 lux (Tukey post hoc test $P = 0.911$). Male body mass negatively affected successful amplexus latency ($X^2_1 = 4.58$, $P = 0.032$). However, this variable was not affected by female body mass, by male salivary testosterone concentrations, by the interaction between light treatment and male body mass, nor by the interaction between light treatment and male

salivary testosterone concentrations. Second, pairs with a control male never split after the amplexus was initiated, whereas the separation rate was by 20% and 10% for males exposed to 0.1 or 5 lux, respectively ($X^2_2 = 5.99$, $P = 0.050$). Even if the model indicated a marginal effect for the chosen significance threshold, the effect size was strong (Cohen's test $|h| = 0.795$), reflecting a clear biological difference. The marginal effect may likely due to low statistical power. A stronger effect may have been observed with more replicates. We did not observe an effect of male body mass, of female body mass, of male salivary testosterone concentrations, of the interaction between light treatment and male body mass or of the interaction between light treatment and male salivary testosterone concentrations on the separation rate. The time spent in amplexus before egg laying was not altered by light treatment, as pairs spent on average 55 ± 4 hours (mean \pm SEM) in amplexus before egg laying. We neither observed an effect of male body mass, female body mass, nor of the interaction between male body mass and light treatment on the time spent in amplexus before egg laying (see Appendix Supplementary Material S4 for details).

ALAN did not modify male salivary testosterone concentrations

After 12 days of exposure, the mean salivary testosterone concentration of the control group was 3.7 ± 0.69 pg mg⁻¹ of saliva (mean \pm SEM). Exposure to ALAN did not modify male salivary testosterone concentrations. We neither observed an effect of male body mass nor of the interaction between male body mass and light treatment on testosterone levels (see Appendix Supplementary Material S4 for details).

Exposure to ALAN reduced male fertilisation success

(Fig. 2)

291

292 When all light treatments were combined, the average clutch size laid by a female was $3347 \pm$
293 272 eggs (mean \pm SEM). Male fertilisation success was affected by the interaction between
294 the male body mass and light treatment (Fig. 2; $X^2_2 = 248.5$, $P < 0.001$). By visually
295 interpreting the difference of slopes, we noticed that in the control and 5 lux groups, the
296 relationship between the fertilisation rate and male body mass was weakly negative, whereas
297 in the 0.1 lux group, this relationship was weakly positive. Moreover, control males had an
298 average fertilisation rate of $75.14 \pm 6.23\%$ (mean \pm SEM), while a 25%-decrease was
299 highlighted in the 5 lux-group compared with control males (Tukey post hoc test $P < 0.001$).
300 A difference between the 0.1 lux- and 5 lux-exposed males was also observed (Tukey post
301 hoc test $P < 0.001$).

302 Discussion

303 Here, we demonstrated that exposure to low light intensities at night during the breeding
304 period alters both the mating behaviours and the fertilisation success of common toad males.
305 Indeed, ALAN affected mating behaviour as male common toads previously exposed to
306 ALAN needed several attempts to maintain a female in amplexus until clutch laying, although
307 all males finally paired in our experimental setup. In line with this first result, the latency to
308 pair successfully with a female was strongly increased by previous exposure to ALAN as well
309 as male body mass. Similarly, *Drosophila melanogaster* reared under 10 lux took longer to
310 initiate mating than control flies (McLay et al. 2018). In the wild, male-male competition is
311 strong in common toad with values of operational sex ratio as high as 4 or 5 males to one
312 female (Davies & Halliday 1979). Therefore, ALAN might increase male-male competition as
313 females will be caught and released several times before one male manages to stay in
314 amplexus until egg laying is complete. The strong operational sex ratio should probably

prevent males that release females from having another possibility to gain a female during the breeding season. In accordance with the important role of photoperiod as a cue to initiate the reproductive season in many species, most studies have shown that ALAN exposure advances the date of reproduction (Ikeno et al. 2014; Dominoni et al. 2016; Le Tallec et al. 2016). Other direct effects of artificial light on sexual behaviour have also been highlighted in various species. For example, Botha et al. (2017) reported that Australian black field crickets, *Teleogryllus commodus*, reared under a very high light intensity (100 lux) were more likely to mate than individuals reared under lower light (0, 1 and 10 lux). Further, male Blue tits, *Cyanistes caeruleus*, occupying territories exposed to streetlights (> 0.06 lux) were twice as successful in obtaining extra-pair mates as males occupying territories further away inside the forest, but no information is given about their breeding success with their social partners (Kempnaers et al. 2010). In addition, ALAN reduced the proportion of mated females Winter moth, *Operophtera brumata*, compared with control females, and the reduction was strongest under green ALAN than red ALAN (Van Greffen et al. 2015). These observations suggest that ALAN may differentially affect mating behaviour depending on animal species and on spectral composition. In our study, we used white cold LED, that emit a prominent peak of blue wavelength. It is well known that many species, including amphibians (Hailman & Jaeger 1974), are sensitive to blue wavelength light, which provokes behavioural and physiological modifications (Musters et al. 2009). It would therefore, be highly interesting to study the effects of low colour temperature and other spectra on the fitness of amphibians.

ALAN negatively affected male fertilisation success, a direct measurement of the breeding component of male fitness that does not provide information on offspring quality but instead informs on their potential number. Present data showed that large males may be more sensitive to high light intensity since under 5 lux, the fertilisation rate was 25% lower than in controls and decreased as body mass rose. The weak negative relationship between

fertilisation rate and male body mass in the controls contrasts with earlier studies showing either a positive or no relationship between fertilisation rate and the ratio between male and female size in wild common toads (Davies & Halliday 1977; Lengagne et al. 2007). The mechanism underlying the deleterious effect of ALAN in large males is unclear. Interestingly, it was suggested that the energetic cost of amplexus increases with size in an anuran species (McLister 2003). Furthermore, we also noticed an effect of body mass on the ALAN-induced rise in basal metabolic rate of common male toads. In males exposed to 5 lux during the night, the largest males showed the highest increase in metabolic rate (Touzot, unpublished data). Further investigations are needed to clarify the link between amplexus cost and fertilisation rate. In amphibian species, large males often have more opportunities to access reproduction than small males (Davies & Halliday 1977). By altering behaviour and fertilisation success, especially in larger males, ALAN may lead to an increase in the genetic contribution of poor-quality males in populations naturally exposed to ALAN. Another scenario could be that larger males would win the competition for female access, but as being the most affected by ALAN, fail at fertilisation, and thus contribute to the reduction in the overall reproductive success of the population.

The physiological mechanisms responsible for the 25% reduction in fertilisation rate in male exposed to 5 lux at night as compared with control are unknown but may be linked to spermatogenesis. Effectively, several studies have reported that exposure to continuous light causes spermatocyte degeneration (Rastogi 1976; Biswas et al. 1978). It should however be noted that in anurans, spermatogenesis lasts 5 or 6 weeks on average (Jørgensen 1984). With regard to the rather short duration of ALAN exposure used in our protocol (males have been exposed to ALAN for 12 days only), a marked deleterious effect on spermatogenesis would be limited. Another possibility is that the fertilisation rate decrease can likely be explained by an alteration of reproductive hormone synthesis. In vertebrates, the effects of ALAN occur at

all levels of the hypothalamic-pituitary-gonadal axis, leading to variations in testosterone levels (reviewed in Ouyang et al. 2018; Brüning et al. 2018). Testosterone would not be involved in the reduced fertilisation rate of common toads as ALAN did not modify salivary testosterone levels. Present observation in toads therefore contrasts with results in birds in which ALAN modified the testosterone secretion pattern during a breeding period (Dominoni et al. 2013) and increased corticosterone levels (Ouyang et al. 2015). The quality of females paired with males cannot be an explanation for our results since females were not subjected to ALAN and we have controlled that female body mass and the ratio between male and female size did not statistically differ among light treatments, thus excluding a female effect. Finally, an alteration of the synchronization of gametes released and/or a reduction in the amount of sperm released by the male can likely explain the fertilisation rate reduction. Indeed, as in all external fertilisation situations, the male must synchronize its sperm release so that it coincides with egg release (Davies & Halliday 1977). Since males exposed to ALAN show increased basal metabolic rate but reduced activity-related energy expenditure (Touzot et al. 2019), sperm emission that requires smooth muscle contractions might also be impaired.

Conclusion

Although some studies have investigated the effects of ALAN on the mortality component of fitness (Rodríguez et al. 2014; Van Doren et al. 2017; Willmott et al. 2018), reports of effects on the breeding component are still scarce, especially in amphibian species. This study showed that relevant nocturnal artificial light intensities can have a major effect on the breeding component of fitness of animals in the wild. Even if a reduction in fertilisation rate was observed at the rather high intensity of 5 lux, the effect although weaker probably occurs at lower light intensities. The separation rate during amplexus, for instance, was affected at an intensity as low as 0.1 lux, which is equivalent to a full moon. If extrapolated to habitats exposed to human activity-related ALAN, present experimental findings suggest that many

toad populations in Europe are expected to incur reproductive losses in their breeding habitats. A better mechanistic understanding of the effects of ALAN, particularly on reproduction, is necessary to predict long term consequences on wildlife and propose appropriate management solutions in an increasingly lighted world.

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Fig. 1. Successful amplexus latency (hour) of males previously exposed for 12 days to 0.01 (black), 0.1 (grey) or 5 (white) lux (horizontal line: median value; box ends: upper and lower quartiles; whiskers: upper and lower 1.5 x interquartile range (IQ); dot: upper dot beyond upper 1.5 x IQ). * < 0.005 ** < 0.001.

Fig. 2. Fertilisation rate (%) of males previously exposed for 12 days to 0.01 (black dots and solid line), 0.1 (grey dots and dashed line) or 5 (white dots and dotted line) lux depending on their body mass. Points represent observed data and the lines represent the predictions of the statistical model (see Statistical Analysis). Shaded areas represent SE calculated from the statistical model.

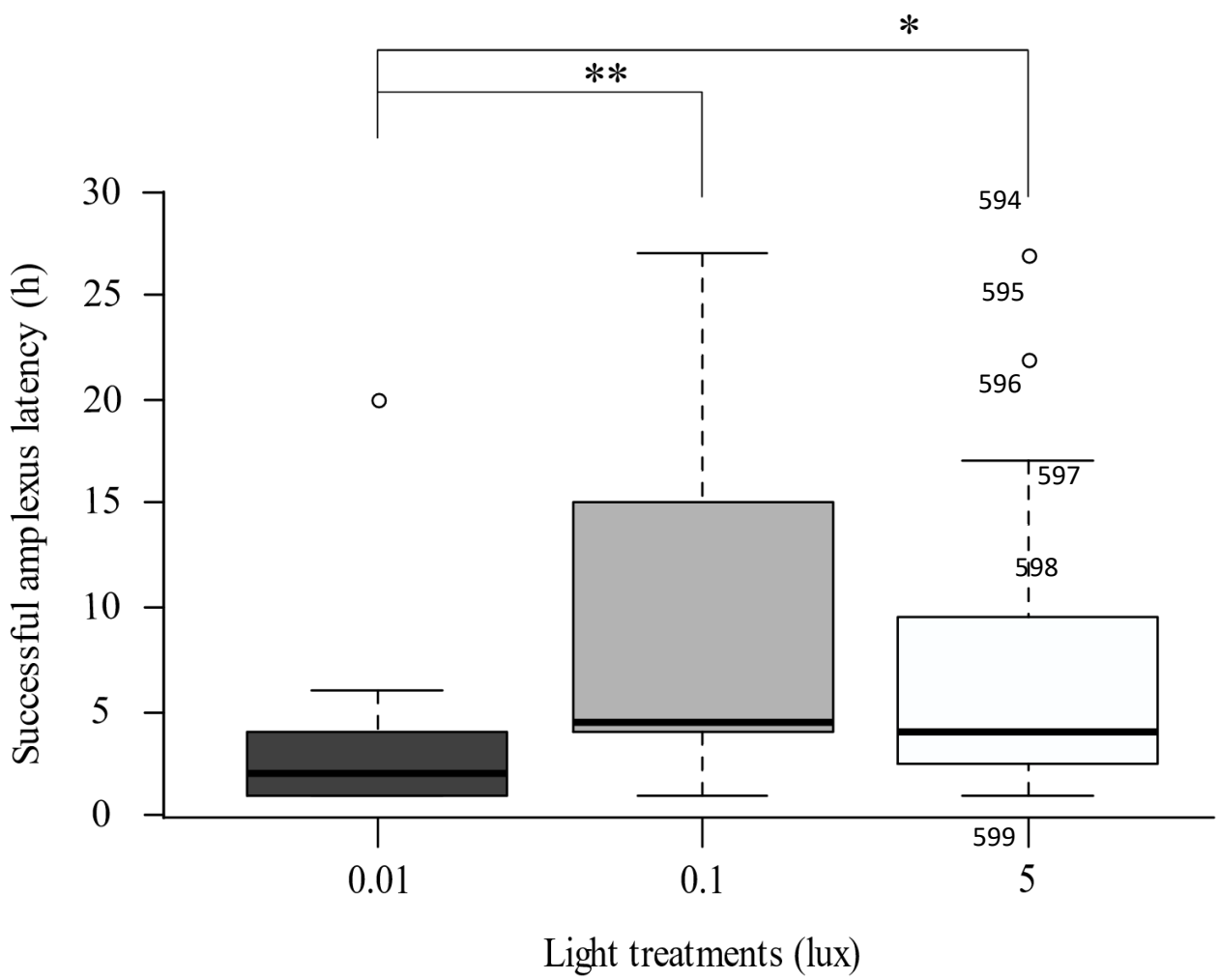


Fig. 1.

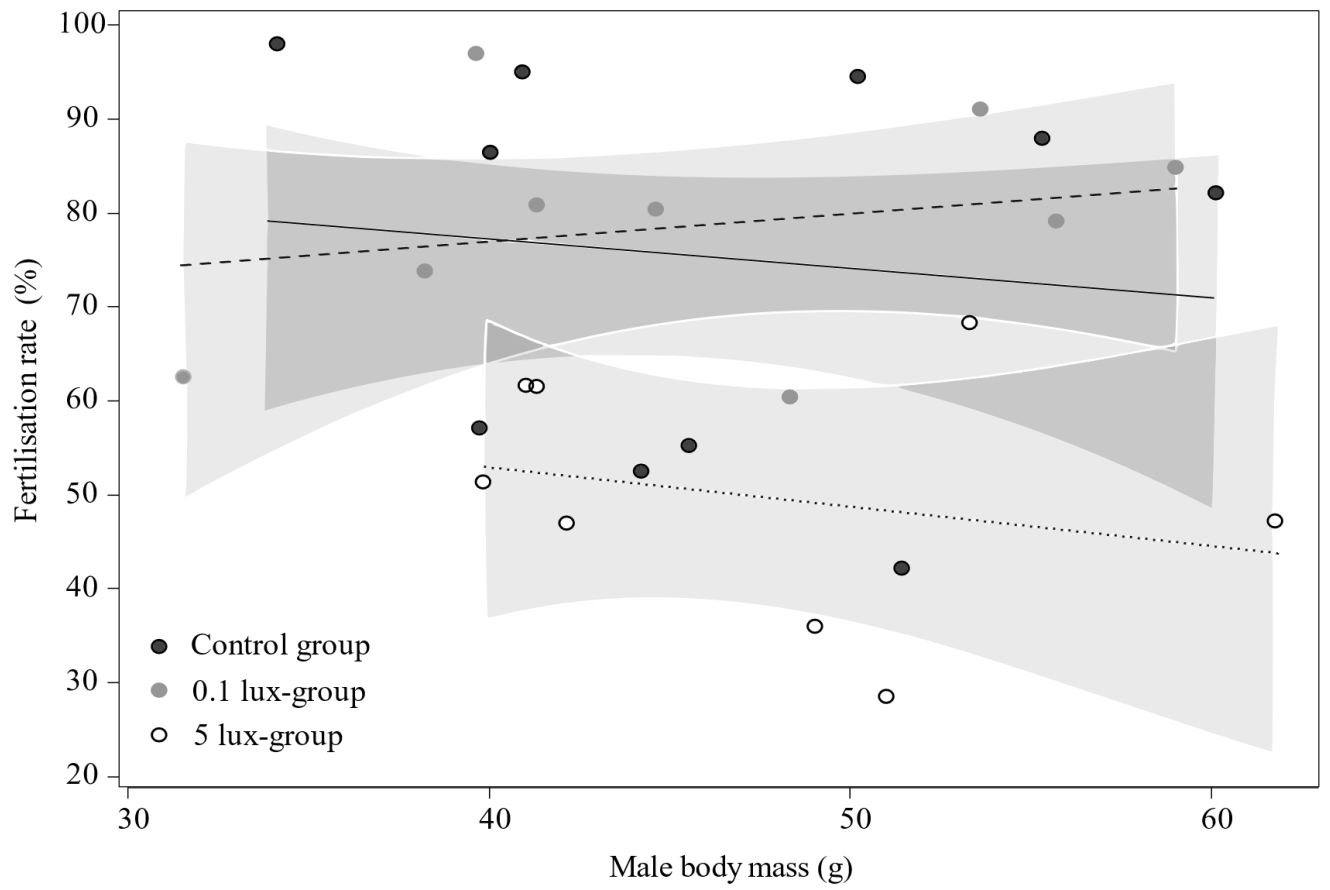


Fig. 2.