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2 toad

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4 **Morgane Touzot^{1,*}, Thierry Lengagne¹, Jean Secondi^{1,2}, Emmanuel Desouhant³, Marc**
5 **Théry⁴, Adeline Dumet¹, Claude Duchamp¹ and Nathalie Mondy¹**

6 ¹Univ Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR 5023 LEHNA,
7 Villeurbanne, F-69622, France.

8 ² Faculté des Sciences, Université d'Angers, 49045 Angers

9 ³Univ Lyon, Université Claude Bernard Lyon 1, CNRS, UMR5558 LBBE, Villeurbanne, F-
10 69622, France.

11 ⁴Centre National de la Recherche Scientifique, Muséum National d'Histoire Naturelle
12 (MNHN), UMR 7179, Brunoy, F-91800, France.

13

14 ***Corresponding author:** Univ Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE,
15 UMR5023 LEHNA, F-69622, Villeurbanne, France. Fax: (+33) 472 431 141, Tel.: (+33)
16 472 431 520. Email: morgane.touzot@univ-lyon1.fr

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21 **Abstract**

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

44 **Capsule summarizing the main finding:** Artificial light at night decreased the capacity of
45 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

47 **Introduction**

48 In recent decades, the rapid growth in the world's population has led to a sharp increase in
49 human activities necessary to support this growth. One consequence of the expansion of urban
50 areas along with the development of transport infrastructures (Grimm et al. 2008; Gaston et
51 al. 2013) is the major increase of light levels at night. In 2016, nearly 23% of the Earth's
52 surface, 88% of Europe and almost half of the United States experienced brightness levels
53 higher than light levels at night in natural ecosystems (Falchi et al. 2016). Artificial Light At
54 Night (ALAN) dramatically expanded. From 2012 to 2016, Earth's artificially lit outdoor area
55 grew by 2.2% per year, with a total radiance increase of 1.8% per year (Kyba et al. 2017).
56 One of the major effects of ALAN is the disruption of the natural photoperiod, which is one
57 of the most important cues for biological timing (Bradshaw & Holzapfel 2010). Among all
58 organisms, nocturnal species, which represent a large proportion of biodiversity, 28% of
59 vertebrates and more than 60% of invertebrates (Hölker et al. 2010), are most likely to
60 experience and to be affected by ALAN (Buchanan 2006; Duffy et al. 2015; Desouhant et al.
61 2019). Nocturnal artificial light is known to affect a wide range of physiological and
62 behavioural phenomena, such as migration (Van Doren et al. 2017), orientation (Tuxbury &
63 Salmon 2005), activity (*e.g.* Le Tallec et al. 2013; Pulgar et al. 2019; Touzot et al. 2019),
64 foraging (Czarnecka et al. 2019), energy balance (*e.g.* Welbers et al. 2017; Touzot et al. 2019)
65 and hormonal synthesis (*e.g.* Brüning et al. 2015; Newman et al. 2015). These effects on
66 individuals have the potential to alter population dynamics (*e.g.* Gaston et al. 2014a; Grubisic
67 et al. 2017; Sanders et al. 2018).

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

72 Fitness, *i.e.* the average contribution to the gene pool of the next generation that is made by
73 individuals of a specified genotype or phenotype in a given environment, is often estimated
74 through measurements of mortality and breeding success. Most of the studies conducted to
75 date focused on the effects of ALAN on the mortality component of fitness (*e.g.* Rodríguez et
76 al. 2014; Willmott et al. 2018). The breeding component of fitness has been less examined,
77 even if However, observation-based studies did not control for other environmental factors
78 that are often correlated with ALAN in the wild (temperature of urban environment, habitat
79 fragmentation, prey availability...), while most experiment-based studies used higher
80 intensities than those actually encountered by individuals in their habitat (May et al. 2019). a
81 growing number of studies (reviewed in Ouyang et al. 2018) reported that the timing and the
82 physiology of seasonal reproductive processes differ between individuals living in lit areas
83 and their conspecifics living in darker areas. Alterations of the period of functional
84 development of reproductive organs, reproductive hormonal synthesis (estradiol,
85 ketotestosterone), number of eggs produced by females, egg hatchling success, number of
86 offspring and birth schedule in response to ALAN exposure were found in mammals (*e.g.* Le
87 Tallec et al. 2015; Robert et al. 2015), birds (*e.g.* Dominoni et al. 2013; de Jong et al. 2015),
88 fishes (*e.g.* Brüning et al. 2018, Fobert et al. 2019) and insects (*e.g.* Willmott et al. 2018;
89 McLay et al. 2018). Photoperiod modifications have also been shown to affect sexual
90 maturation and sex steroids synthesis, especially in fish (*e.g.* **Norberg et al. 2004**). The
91 suppression of the dark period triggered a decrease of spermatocytes in male Asian toads,
92 *Bufo melanostictus*, (Biswas et al. 1978) and a severe reduction of sexual calls in male green
93 frogs, *Rana clamitans melanota*, (Baker & Richardson 2006). On contrary, other studies have
94 found no effect of ALAN on the time for copulation or between mating and laying egg, on the
95 number of eggs or egg sacs laid and on sperm viability (*e.g.* Durrant et al. 2018; McLay et al.
96 2018; Willmott et al. 2018). A recent study investigating the influence of ALAN on the

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

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

121 **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

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

169 *Reproductive behaviours*

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

188 *Hormonal assay*

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

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

206 *Statistical Analysis*

207 We performed a linear model (LM) to test the effects of the light treatment (0.01, 0.1, 5 lux)
208 on male body mass gain during ALAN exposure and on male body mass on D13. To confirm
209 that there were no confounding differences in body size between light treatments, we
210 performed a LM to test the effect of light treatment (0.01, 0.1, 5 lux) on male SVL
211 measurement and on the ratio between male and female size on D13. Successful amplexus
212 latency data were analysed as survival data following a Weibull distribution. Light treatment
213 (0.01, 0.1, 5 lux), male body mass, female body mass, male salivary testosterone
214 concentrations, and the interactions between light treatment and male body mass and between
215 light treatment and male salivary testosterone concentrations were included as explanatory
216 variables. The effect of light treatment (0.01, 0.1, 5 lux), male body mass, female body mass,
217 male salivary testosterone concentrations and the interactions between light treatment and
218 male body mass and between light treatment and male salivary testosterone concentrations on
219 the probability of separation before egg laying (separation = 1; no separation = 0) was

220 analysed through a generalized linear model (GLM) with a binomial distribution (logit link).
221 As the results of the model indicated a marginal effect for the chosen significance threshold
222 (5%), we calculated the effect size (Cohen's h for proportions). We performed a GLM with a
223 Gamma distribution to test the effect of the light treatment (0.01, 0.1, 5 lux), male body mass,
224 female body mass and the interaction between light treatment and male body mass on
225 amplexus duration before egg laying. For this analysis, one value is missing in the 5 lux-
226 group, as one female paired to a 5 lux-exposed male did not lay eggs. We performed a LM to
227 test the effects of the light treatment (0.01, 0.1, 5 lux) and male body mass and their
228 interaction on male salivary testosterone concentrations. For male salivary testosterone
229 concentrations, one data point was excluded because the value was below the colour range of
230 the ELISA kit. Male fertilisation rate was analysed through a GLM with a binomial
231 distribution (logit link) using male body mass, female body mass, light treatment and the
232 interaction between light treatment and male body mass as predictors. Because clutches are
233 synchronous and, given the number of eggs to be counted, we counted fertilised eggs on a
234 sub-sample of pairs ($n = 10$ for control, $n = 9$ for 0.1 lux and $n = 8$ for 5 lux), which
235 corresponds to 94 102 eggs counted. For the analyses, the fitted models were simplified by a
236 backward procedure to keep only the significant variables. For all models, multiple
237 comparisons were performed using a Tukey adjusted post hoc test. All data were analysed
238 using the software R 3.5.1 (R Core Team 2018) and the packages *lsmeans* (for successful
239 amplexus latency and male fertilisation rate analysis), *pwr* (for the probability of separation
240 before egg laying) and *survival* (for successful amplexus latency analysis).

241 *Ethical note*

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

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

247 **Results**

248 **ALAN affected reproductive behaviours of males**

249

250 **(Fig. 1)**

251

252 Male body mass gain during ALAN exposure was on average of 10.66 ± 1.27 g in the control
253 group (mean \pm SEM) and did not significantly differ between the light treatments (see
254 Appendix Supplementary Material S4 for details). At mating, on D13, male body mass, male
255 SVL measurement and the ratio between male and female size did not significantly differed
256 between the light treatments (see Appendix Supplementary Material S4 for details). For all
257 light treatments, each male performed an amplexus with the female. However, ALAN
258 strongly affected the reproductive behaviours of male common toads. First, successful
259 amplexus latency significantly differed between pairs with an exposed male and pairs with a
260 control male (Fig. 1; $X^2_2 = 14.01$, $P < 0.001$). Males exposed to 0.1 or 5 lux took much longer
261 to achieve successful amplexus as shown by the 3-fold increase (Tukey post hoc test $P <$
262 0.001) and the 1.5-fold increase in latency (Tukey post hoc test $P = 0.004$), respectively,
263 compared with control males. No significant difference was found between males exposed to
264 0.1 or 5 lux (Tukey post hoc test $P = 0.911$). Male body mass negatively affected successful
265 amplexus latency ($X^2_1 = 4.58$, $P = 0.032$). However, this variable was not affected by female
266 body mass, by male salivary testosterone concentrations, by the interaction between light
267 treatment and male body mass, nor by the interaction between light treatment and male

268 salivary testosterone concentrations. Second, pairs with a control male never split after the
269 amplexus was initiated, whereas the separation rate was by 20% and 10% for males exposed
270 to 0.1 or 5 lux, respectively ($X^2_2 = 5.99$, $P = 0.050$). Even if the model indicated a marginal
271 effect for the chosen significance threshold, the effect size was strong (Cohen's test $|h| =$
272 0.795), reflecting a clear biological difference. The marginal effect may likely due to low
273 statistical power. A stronger effect may have been observed with more replicates. We did not
274 observe an effect of male body mass, of female body mass, of male salivary testosterone
275 concentrations, of the interaction between light treatment and male body mass or of the
276 interaction between light treatment and male salivary testosterone concentrations on the
277 separation rate. The time spent in amplexus before egg laying was not altered by light
278 treatment, as pairs spent on average 55 ± 4 hours (mean \pm SEM) in amplexus before egg
279 laying. We neither observed an effect of male body mass, female body mass, nor of the
280 interaction between male body mass and light treatment on the time spent in amplexus before
281 egg laying (see Appendix Supplementary Material S4 for details).

282 **ALAN did not modify male salivary testosterone concentrations**

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

288 **Exposure to ALAN reduced male fertilisation success**

289

290 **(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

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

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

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

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

365 all levels of the hypothalamic-pituitary-gonadal axis, leading to variations in testosterone
366 levels (reviewed in Ouyang et al. 2018; Brüning et al. 2018). Testosterone would not be
367 involved in the reduced fertilisation rate of common toads as ALAN did not modify salivary
368 testosterone levels. Present observation in toads therefore contrasts with results in birds in
369 which ALAN modified the testosterone secretion pattern during a breeding period (Dominoni
370 et al. 2013) and increased corticosterone levels (Ouyang et al. 2015). The quality of females
371 paired with males cannot be an explanation for our results since females were not subjected to
372 ALAN and we have controlled that female body mass and the ratio between male and female
373 size did not statistically differ among light treatments, thus excluding a female effect. Finally,
374 an alteration of the synchronization of gametes released and/or a reduction in the amount of
375 sperm released by the male can likely explain the fertilisation rate reduction. Indeed, as in all
376 external fertilisation situations, the male must synchronize its sperm release so that it
377 coincides with egg release (Davies & Halliday 1977). Since males exposed to ALAN show
378 increased basal metabolic rate but reduced activity-related energy expenditure (Touzot et al.
379 2019), sperm emission that requires smooth muscle contractions might also be impaired.

380 **Conclusion**

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

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

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

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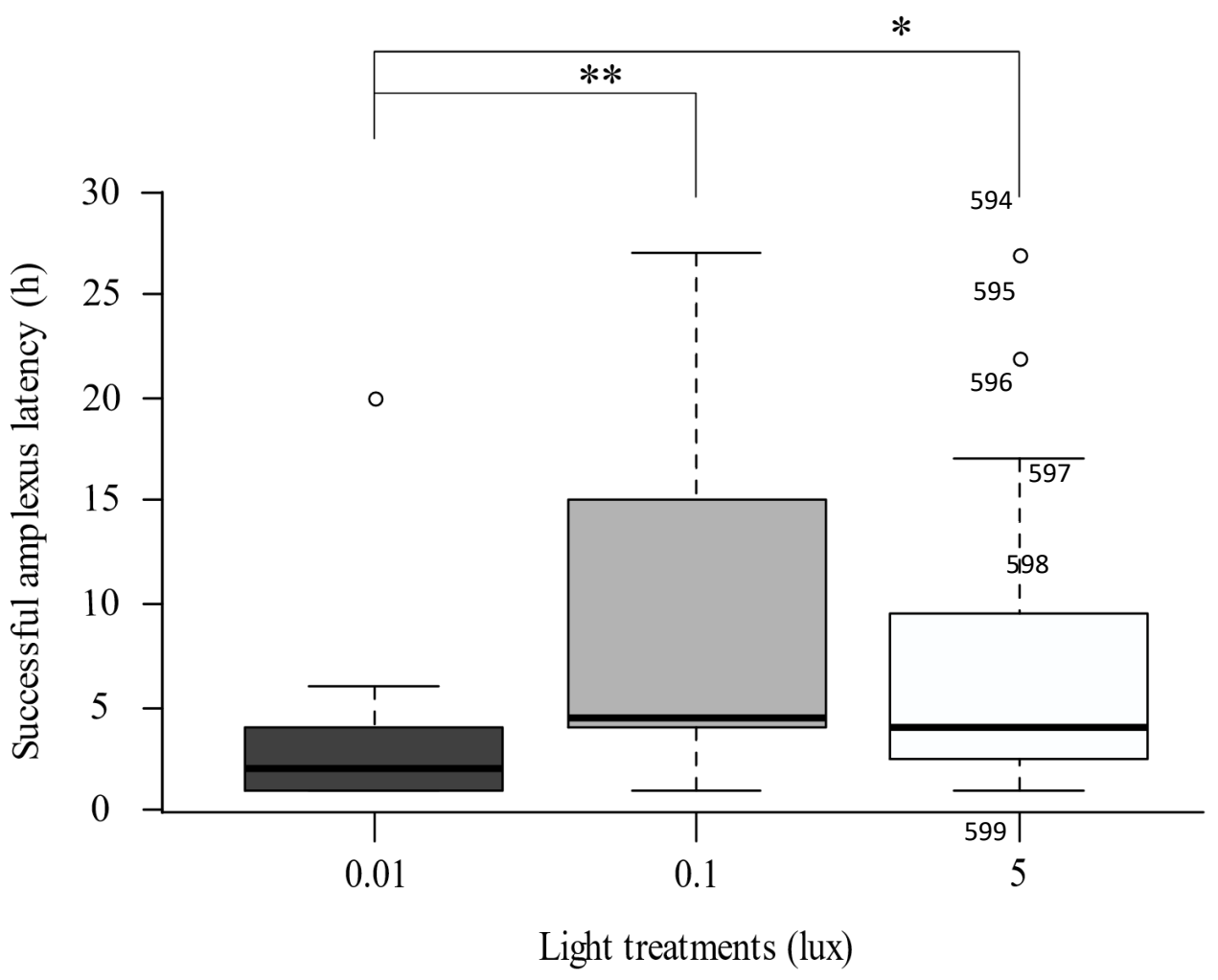
585 **Fig. 2.** Fertilisation rate (%) of males previously exposed for 12 days to 0.01 (black dots and
586 solid line), 0.1 (grey dots and dashed line) or 5 (white dots and dotted line) lux depending on
587 their body mass. Points represent observed data and the lines represent the predictions of the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

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602 **Fig. 1.**

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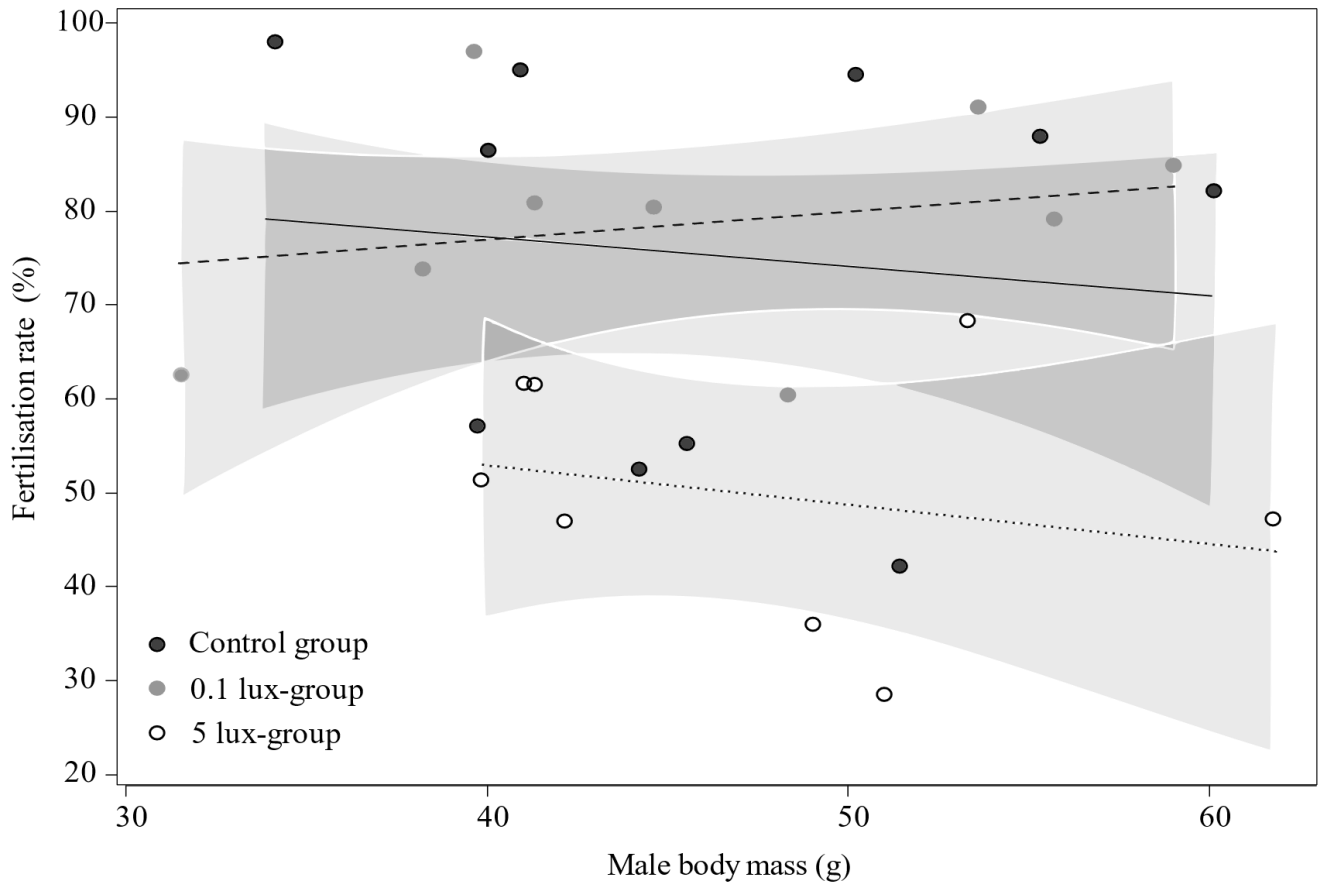
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613 **Fig. 2.**