



Ulgezen, Z. N., Käpylä, T., Meerlo, P., Spoelstra, K., Visser, M. E. and Dominoni, D. M. (2019) The preference and costs of sleeping under light at night in forest and urban great tits. *Proceedings of the Royal Society of London Series B: Biological Sciences*, 286(1905), 20190872.

There may be differences between this version and the published version. You are advised to consult the publisher's version if you wish to cite from it.

<http://eprints.gla.ac.uk/189211/>

Deposited on: 20 August 2019

Enlighten – Research publications by members of the University of Glasgow_
<http://eprints.gla.ac.uk>

21 **Abstract**

22 Artificial light at night (ALAN) is an increasing phenomenon associated with worldwide
23 urbanisation. In birds, broad-spectrum white ALAN can have disruptive effects on activity
24 patterns, metabolism, stress response and immune function. There has been growing research
25 on whether the use of alternative light spectra can reduce these negative effects, but
26 surprisingly, there has been no study to determine which light spectrum birds prefer. To test
27 such a preference, we gave urban and forest great tits (*Parus major*) the choice where to roost
28 using pairwise combinations of darkness, white or green dim light at night (1.5 lux). Birds
29 preferred to sleep under artificial light instead of darkness, and green was preferred over white
30 light. In a subsequent experiment, we investigated the consequence of sleeping under a
31 particular light condition, and measured birds' daily activity levels, daily energy expenditure
32 (DEE), oxalic acid as a biomarker for sleep debt, and cognitive abilities. White light affected
33 activity patterns more than green light. Moreover, there was an origin-dependent response to
34 spectral composition: in urban birds the total daily activity and night activity did not differ
35 between white and green light, while forest birds were more active under white than green
36 light. We also found that individuals who slept under white and green light had higher DEE.
37 However, there were no differences in oxalic acid levels or cognitive abilities between light
38 treatments. Thus, we argue that in naïve birds that never encountered light at night, white light
39 might disrupt circadian rhythms more than green light. However, it is possible that negative
40 effects of ALAN on sleep and cognition might be observed only under intensities higher than
41 1.5 lux. These results suggest that reducing the intensity of light pollution as well as tuning the
42 spectrum towards long wavelengths may considerably reduce its impact.

43 **Introduction**

44 Light pollution refers to the diminishing of darkness during night-time, caused by light from
45 anthropogenic sources. Artificial light at night (ALAN) can threaten ecosystem dynamics
46 through alterations in the biological timing of a wide range of species, with far-reaching
47 consequences [1,2]. For instance, ALAN can lead to lethal consequences due to attraction to
48 light sources, such as for hatching sea turtles [3] and migrating birds [4]. Night-time
49 illumination can also have more subtle effects through changes in physiological processes and
50 behaviour due to disruption of natural circadian rhythms and sleep, which in turn may affect
51 the individual's health and ultimately fitness [1].

52 Birds use light cues for synchronising their biological rhythms [5] and ALAN can alter
53 their photoperiodic perception [6–9]. Consequently, ALAN can affect the timing of
54 reproductive physiology and behaviour [8,10,11], timing of dawn singing [10,12,13] and sleep
55 behaviour [14] of free-living passerine songbird species. Experimental studies on captive
56 songbirds have confirmed work in the wild[7,15]. Blackbirds increase locomotor activity at
57 night when roosting under light compared to darkness [15]. Similarly, great tits advance
58 activity, delay activity offset, and move a higher proportion of their daily activity into the night
59 when exposed to ALAN [7].

60 Although there is increasing evidence that ALAN alters biological rhythms, the
61 consequences of such alteration are not always fully understood. ALAN can decrease
62 melatonin production at night [20], increase blood inflammatory markers [21] and increase
63 susceptibility to pathogens [22]. However, the increase in nocturnal activity due to ALAN
64 could have a major impact also on energy consumption and sleep [16,17]. Energy is a crucial
65 and limited resource for animals, and there is a trade-off between investment decisions on
66 behavioural and/or physiological processes and these trade-offs are often associated with
67 fitness [18]. A measurement of energy metabolism is daily energy expenditure (DEE). While

68 DEE is mostly affected by body mass [19], it can also be influenced by environmental factors
69 such as human disturbance, temperature and food availability [18,19]. In the context of light
70 pollution, higher activity at night due to ALAN could potentially increase the energy
71 expenditure of diurnal animals, with carry-over consequences on other physiological systems
72 as well as fitness. However, in a recent field study on great tits (*Parus major*), we showed that
73 a lower DEE was related to breeding in territories illuminated with white and green lights
74 compared to dark areas [20]. This decrease in DEE could be explained by other ecological
75 factors, such as the increase in food availability (insects) in artificially illuminated areas [20].
76 Furthermore, in forest areas, birds can avoid artificial lighting by choosing a distant nesting
77 location [21], thereby possibly evading the negative effects of nocturnal light. As such, the
78 direct effects of artificial light on DEE are yet unknown.

79 Other potential ecological costs of ALAN might arise through loss of sleep, as shown
80 in humans [22]. Indeed, in birds previous studies suggested that ALAN is associated with
81 nocturnal restlessness, that is, activity bouts that are clearly distinguishable from sleep
82 behaviour. For instance, female great tits exposed to ALAN for two nights in nest boxes slept
83 less and had shorter sleep bouts compared to birds who roosted under darkness [14]. However,
84 such short-term manipulation appeared to be transient as birds showed regular sleep behaviour
85 when the exposure to light at night was stopped. Moreover, it is unclear whether such nocturnal
86 restlessness really represents sleep disruption. Recently, reduced plasma levels of oxalic acid
87 have been established as a biomarker of sleep disruption in humans and rodents [23]. This
88 opens the possibility to measure sleep disruption in non-model organisms in the field. A recent
89 study in great tits showed that a higher nocturnal activity due to ALAN was associated with a
90 decrease in oxalic acid, thereby suggesting a negative effect of ALAN on sleep [24]. Sleep is
91 a key state for the consolidation of memory, and thereby affects information use [25].
92 Information processing, retention and use is a part of cognition and important for behavioural

93 decision-making processes, and cognitive abilities allow animals to detect danger, remember
94 food resources and nesting sites based on environmental cues [25]. Studies with great tits show
95 that they are able to memorise locations of cached food by observing other bird species and
96 steal resources, indicating the importance of cognition on fitness [26]. In birds, cognition is
97 affected by sleep, and thus cognitive abilities may be altered by sleep disruption due to ALAN
98 [16]. In a recent study, birds kept under constant daylight showed a disruption in their activity
99 patterns and a deterioration in their cognitive performance [27]. However, the effects of dim
100 rather than constant bright ALAN on cognition remain unknown.

101 Although ALAN is increasingly associated with negative ecological effects, it is also
102 necessary in human society for economic and safety reasons. Currently there is an increase in
103 the use of broad-spectrum light emitting diode (LED) lamps due to their cost-effectiveness
104 [28]. As LED lights can easily be adjusted to different light spectra, this may offer the
105 possibility of using different light spectrum to decrease the ecological negative impact of light
106 pollution. In birds, broad-spectrum white LED light seems to have major impacts, such as
107 altering immune response [24], advancing reproductive activities [29] and increasing
108 corticosterone levels [30] compared to control birds not exposed to ALAN. Experiments with
109 blue tits (*Cyanistes caeruleus*) have shown that, at lower intensities, green light is less
110 disruptive (compared to white and red light) on activity patterns [31].

111 While the effects of different light colours are yet to be fully appreciated, it is also
112 unclear whether animals would prefer any type of light spectra when selecting for a roosting
113 location, everything else being equal. Animals generally make behavioural decisions that
114 maximizes their fitness, and therefore should choose for environments that satisfy their
115 requirements the most [32]. On one hand animals might benefit from roosting in lit areas
116 because they could forage at night, but on the other hand they could suffer from increased
117 predation risk and sleep disruption. These trade-offs may be modulated by light intensity and

118 spectra. There has been some research in the poultry sector about the preference of chickens
119 for artificial light of different colours, which showed that these birds seem to prefer light with
120 high colour temperature (spectra) [33]. However, these studies were not carried out in the
121 context of nocturnal lighting. Furthermore, even closely related species can show behavioural
122 differences with regards to ALAN [34], and thus it is difficult to make generalisations. As of
123 yet, there has not been any research into whether wild bird species prefer to roost in dark vs lit
124 areas, and into whether a specific spectrum of artificial light at night would be preferred.

125 The aim of this study is to test the preference of birds for roosting in darkness versus
126 different light spectra, and understand the physiological, behavioural, and cognitive
127 consequences of different spectra of ALAN. In a laboratory setting, we exposed male great tits
128 to green light, white light (at similar intensities of 1.5 lux), or darkness. This light intensity is
129 comparable to what wild birds are exposed to in light polluted areas [11,21] and in captive
130 studies has been shown to have moderate effects on activity patterns of great tits [7]. We chose
131 to use green light because there is considerable interest to find light colours that minimise the
132 effects of light pollution on wildlife, and green has been suggested to be a potential option as
133 it is also suitable for outdoor lighting [35]. We used birds both from urban and rural areas to
134 assess whether urban birds respond differently to night light compared to forest birds, as
135 previous research suggested that prolonged exposure to ALAN might alter sensitivity to light
136 [11]. In a first experiment, we tested the preference of birds by giving them the choice of where
137 to roost between pairwise combinations of darkness, green light or white light. In a second
138 experiment, we forced birds to roost under a specific night light and measured daily activity
139 patterns, DEE, plasma concentrations of oxalic acid, sleep behaviour and cognitive abilities.
140 Our hypothesis is that birds prefer to roost under darkness than any light colour. White light,
141 and to a lesser extent green light, will increase night time activity and cause sleep debt, thereby
142 increasing the DEE and negatively affecting cognitive ability. We also expect that night light

143 will have less disruptive effects on the physiology of urban birds as they might have developed
144 tolerance to the presence of ALAN.

145

146 **Methods**

147 *(a) Birds and housing*

148 We studied 35 (17 forest and 18 urban) male great tits. The birds were caught in the wild (see
149 Electronic Supplementary Material (ESM), Figure S1, for a map of catching locations) and
150 transported to the Netherlands Institute of Ecology (NIOO-KNAW) Wageningen, the
151 Netherlands. Birds were housed in individual cages (90 m x 50 m x 40 m), initially spread over
152 three adjacent rooms. Each cage had two light sources, one for day and one for night. The front
153 of each cage was covered with a wooden board to exclude any external light from the outside
154 and neighbouring cages. Birds were provided with food and water *ad libitum*. Over the course
155 of the first experiment (experiment 1), which was from 9 October until 28 October (21 days),
156 birds had a constant photoperiod of 10.15h light - 13.45h dark, and for the second experiment
157 (experiment 2), which was from November 6 until December 17 (42 days) birds had a constant
158 photoperiod of 8.15h light – 15.45h dark. This was the average of natural daytime and night-
159 time hours throughout the dates that the experiments were carried out.

160

161 *(b) General experimental set-up*

162 In both experiments we used a within-individual design such that all birds were exposed to
163 every treatment. Each experiment consisted of three treatment periods and the order of
164 treatments was randomized across birds and rooms. During the daytime, birds were exposed to
165 full florescent spectral light at ± 1000 lux (Activa 172, Philips), and at night they were exposed
166 to LED lamps with either green light, white light, or darkness (no light). While both green and
167 white lights emit full spectrum light, green lamps have an increased blue and reduced red

168 emission (for spectra see supplement to [31]). Green and white light intensities during night
169 time were set at 1.5 lux, measured at perch level. To ensure that light intensities were the same
170 in all cages we tested lights at perch level with a lux meter before the start of the experiments.
171 Between the two experiments, birds had seven days of recovery period. During this recovery
172 period birds were exposed to dark nights. In both experiments, night lights had a 15-minute
173 overlap with daylights both in the morning and in the evening.

174

175 *(c) Experiment 1: Choice experiment*

176 Experimental set-up

177 Each bird was placed into a combined cage made up of two adjacent individual cages that were
178 connected through a 7 cm diameter hole. Birds were allowed to move freely between the sub-
179 cages (Fig. S1-A). Individuals were assigned randomly to a treatment group and to one of 12
180 blocks of cages divided over three rooms. Each block contained all three treatments and both
181 origins. During the daytime, the conditions of the sub-cages were the same but at night time,
182 the light in the two sub-cages was different. Birds were exposed to one of three treatments:
183 white light-green light (WG), darkness-greenlight (DG) or darkness-white light (DW).
184 Treatments lasted five nights followed by two days of recovery (Fig. S1-B). After the second
185 night of the five-nights treatment, the placement of the light in the sub-cages was switched
186 around to account for the possibility of the bird choosing one sub-cage over the other regardless
187 of the presence or absence of light.

188

189 Light preference

190 Camera traps were used to record the sub-cage chosen by the birds. The cameras were set to
191 take a picture at one-minute intervals as well as to take a picture based on motion detection.
192 While an actual camera was placed in one sub-cage to record preference, a dummy camera was
193 placed in the adjacent sub-cage. The dummy was used to correct for the possibility of the

194 camera to act as a novel object and thereby affect cage choice independent of light. For every
195 night, in each sub-cage the choice of the bird was recorded as a binary response of either yes
196 (bird in cage) or no (bird not present in cage). Birds perched everywhere in the cage during
197 their sleep, including perches, the ground, on top or behind feeders, and even on the hole
198 dividing the two sub-cages. In case of the latter event, given that that the head of the bird was
199 in one cage and the tail in the other, the position of the head was considered the bird's choice,
200 as bird photoreceptors are located in the head (eye, pineal gland and hypothalamus) [5].

201

202 *(d) Experiment 2: Forced light exposure*

203 Experimental set-up

204 In experiment 2, all birds were placed into separate individual cages. The treatments to which
205 the birds were exposed at night were: white light, green light, or darkness. Individuals were
206 randomly placed into a treatment group and to one of six blocks divided over two rooms. Each
207 block contained all three treatments and both origins. Every treatment lasted two weeks (14
208 days), where birds received 11 nights of treatment, followed by three nights of recovery (Suppl.
209 Fig. 1B). Two birds (one forest and one urban) died during the recovery week between the two
210 experiments due to unknown causes (thus $N = 33$).

211

212 Activity measurements

213 Daily activity patterns of each individual bird were measured continuously following the same
214 method described in de Jong et al [7]. We focused on onset of activity, offset of activity, total
215 activity and nocturnal activity. For a more detailed explanation of how the measurements were
216 obtained please see the ESM.

217

218 Nocturnal restlessness

219 We used camera traps for the assessment of slight movements of birds during sleep. Cameras
220 were set to take pictures on motion detection as well as at one-minute intervals. We looked at
221 the time frames between latest activity offset and earliest activity onset to observe sleep
222 behaviour. In every treatment, for each bird one night was selected. We aimed to select the
223 same night (the seventh night after the treatment started) as an observatory unit for each
224 treatment period of two weeks. If that was not possible because the bird was not clearly visible
225 on that night, then we selected the closest available night. The light treatment did not affect the
226 likelihood of a bird being clearly visible in the camera frame ($p>0.1$). To assess sleep
227 behaviour, we went through the night recordings frame by frame. If the bird was in the same
228 sleep position between two subsequent frames, and with the head tucked beneath the shoulder,
229 it was recorded as ‘no movement’ (0), and whenever the bird moved its head or changed
230 sleeping position over the period of time frame, it was recorded as ‘restlessness’ (1). This
231 distinction was based on previous papers that assessed sleep behaviour in great tits. We
232 recognise that sometimes birds might sleep also with the head outside of the feather, especially
233 during REM-related sleep [36], but unfortunately we did not have any mean to distinguish such
234 events without having corresponding EEG recordings.

235

236 Daily energy expenditure

237 DEE of birds was measured in a subsample of 11 birds with the doubly labelled water (DLW)
238 technique through the collection of breath samples, which has been validated in previous
239 studies [20,37]. All 11 birds were measured in each treatment period and thus we obtained a
240 total of 33 DEE measurements. The order of treatments and origins were randomised. A
241 detailed explanation of this procedure can be found in the ESM.

242

243 Oxalic acid

244 Before the start of the experiment 2 and at the end of each 12-day treatment, we took a blood
245 sample (in total four per bird) to measure plasma concentrations of oxalic acid. Details of
246 sampling and laboratory assays are described in [24] and in the ESM.

247
248 Cognitive abilities

249 Cognition was measured with a subsample of 22 birds (11 urban and 11 forest) in experiment
250 2, through learning and memory tasks adapted from the dimensional shift paradigm by Titulaer
251 et al. [38]. A dimensional shift paradigm examines learning ability through behavioural
252 responses to environmental cues. Overall, we tested six tasks, four learning and two memory
253 tasks (see Table S3 for a schematic representation). A detailed explanation of these procedures
254 can be found in the ESM.

255

256 *(e) Statistical Analysis*

257 All data was analysed with SPSS statistics (version 24, IBM SPSS), with a significance level
258 of $\alpha = 0.05$. We used generalized linear mixed-effect models (GLMMs) with logistic regression
259 for binary responses (light preference, cognition, and movement), and for all other response
260 variables we used linear mixed-effects models (LMMs). Assumptions for using linear models
261 were met. Individuals nested within blocks were added into all models as random effects to
262 account for the repeated measurements of birds and location of cages. If an interaction term
263 was significant, we performed post-hoc tests with Bonferroni correction. Model selection was
264 done by step-wise deletion of non-significant terms, starting with the interaction term.

265 In experiment 1, we separated and analysed data per treatment (WG, DW, DG). Night
266 lights in sub-cage (green, white, dark), origin, and position of cage (left/right) were added into
267 the models as fixed effects with interaction of night light*origin. In experiment 2, we ran four
268 separate models to analyse activity patterns. In these models the following four response
269 variables were used: activity onset, activity offset, nocturnal activity and total activity. The

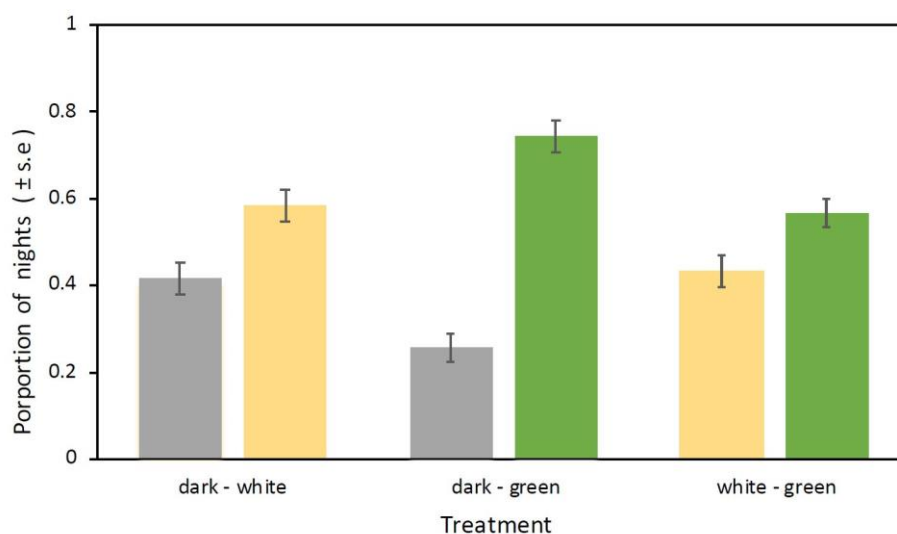
270 three-way interaction of treatment, origin and treatment day (i.e. the days of treatment - night
271 lights - in each treatment period) was initially fitted into all models. In the analysis of DEE,
272 change in oxalic acid and sleep restlessness, we included origin, treatment and their interaction
273 as fixed effects. For cognition, we ran a model with the interaction of type*treatment*origin.
274 Type was defined as the sort of task (memory/learning) birds had to complete in the cognition
275 test. If the interaction was significant we separated data by type.

276

277 **Results**

278 *(a) Experiment 1: Choice Experiment*

279 In all three treatments night light had a major effect on the choice of birds, and green light was
280 the predominant preference (Fig. 1 and Table S1), regardless of the origin of the birds. Birds
281 generally chose to roost under light at night compared to darkness, both in DW ($p = 0.002$) and
282 DG treatments ($p < 0.001$). In WG, birds chose to roost under green light ($p = 0.014$) in
283 comparison to white light. Light position (left/right) only had a minor effect in the WG
284 treatment ($p = .041$), where birds preferred to roost in the cage on the right (estimate = 0.55,
285 $SE = 0.04$) over left.



286

287 **Figure 1.** Proportion of nights spent under different light conditions in pairwise light
288 treatments. Bars and errors bars represent means \pm SEM.

289

290 (b) *Experiment 2: Forced light exposure*

291 Effects of ALAN on activity traits

292 In experiment 2, activity patterns were disrupted by ALAN compared to darkness, and more
293 so for birds roosting under white light, especially for forest birds (Fig. S2 and Table 1). The
294 interaction between origin and treatment had an effect on all variables except activity offset
295 (Table 1). The greatest changes were observed in the activity onset of birds. White light had
296 the most severe effect (urban: estimate = -148 min, $SE = 8.5$; forest: estimate = -158 min, SE
297 = 8.8), advancing onset almost by three hours. Birds roosting under green light also started
298 their day earlier, but to a lesser extent compared to white light (urban: estimate = -123 min, SE
299 = 8.5; forest: estimate = -117 min, $SE = 8.7$). Moreover, there was a significant treatment*origin
300 effect: while urban birds responded more strongly than forest birds to green light, the reverse
301 was true for white light (Table 1 and Fig. S2). The effect of ALAN on activity offset was
302 weaker and did not depend on origin (Fig. S2-B). It was highest for birds roosting under green
303 light (estimate: 31 min, $SE = 3.8$), followed by white light (estimate = 20 min, $SE = 3.9$), and
304 then dark, where offset was close to lights off (estimate = 4 min, $SE = 3.9$). Nocturnal activity
305 was higher in birds exposed to ALAN compared to birds under darkness (DW, DG: $p < 0.001$,
306 Fig. S2-C). For forest birds, there was a significant difference in nocturnal activity between
307 light spectra ($p < 0.001$), because birds were more active under white light (estimate = 118
308 min, $SE = 8.4$) compared to green light (estimate = 86 min, $SE = 8.4$). However, the difference
309 was not significant ($p = 0.08$) for urban birds. Similarly, total activity was higher under ALAN
310 compared to darkness (DW, DG $p < 0.001$, Fig. S2-D). While forest birds had a higher total
311 activity ($p < 0.001$) under white light (estimate = 481 min, $SE = 18.0$) compared to green light
312 (estimate = 442 min, $SE = 18.0$), urban birds showed no difference in total activity with regards
313 to light spectra ($p = 0.07$).

314 There was also a major effect of treatment-day on the activity patterns of birds excluding
 315 nocturnal activity (Table 1). Activity onset advanced over time under both treatments, but
 316 while there were similarities in the first treatment-days, onset declined more so under white
 317 light than under green light (Fig. S2-A). Conversely to onset, activity offset was delayed more
 318 in the first few treatment-days under ALAN, and the offset times became closer to dark nights
 319 over time (Figure S2-B). During the relative night birds did not change activity levels over time
 320 (Fig. S2-C). The total activity measured in a 24-h period increased in the first couple of
 321 treatment-days and then plateaued over time (Fig. S2-D).

322 **Table 1** Results of the LMMs on the four activity response variables in experiment 2. Model outputs
 323 for significant terms are given. The numerator degrees of freedom (ndf), denominator degrees of
 324 freedom (ddf), F-statistic (F), and p-value (p) is given for each term.

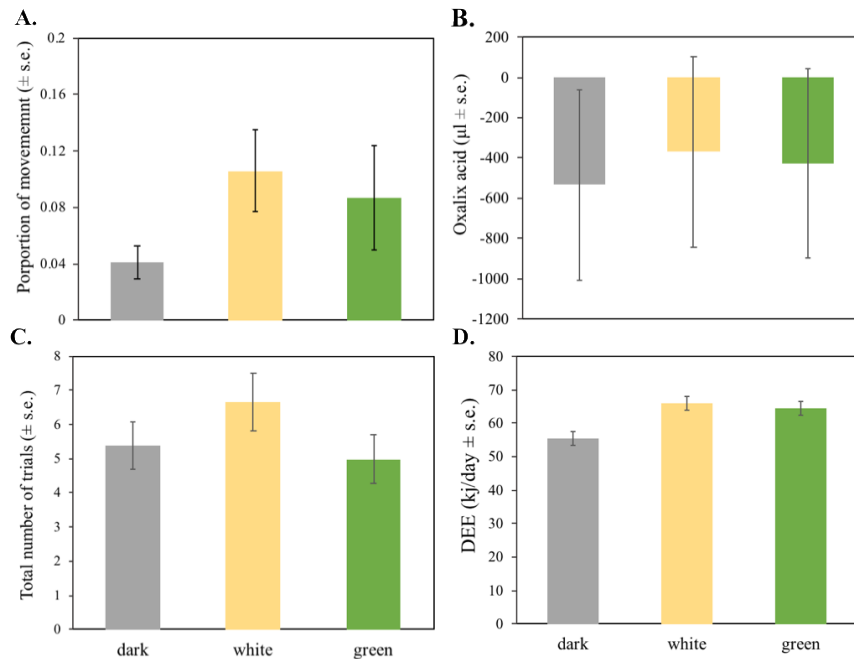
Response	Explanatory	ndf, ddf	F	p
Activity onset	Origin*Treatment	2, 882.1	3.2	0.041
	Treatment* Treatment-day	20, 878.8	4.3	< 0.001
Activity offset	Treatment*Treatment-day	20, 883.6	1.8	0.014
Nocturnal activity	Origin*Treatment	2, 970.9	5.6	0.004
Total activity	Origin*Treatment	2, 969.2	18.1	< 0.001
	Treatment-day	10, 968.2	8.9	< 0.001

325

326 Effects of ALAN on nocturnal restlessness, oxalic acid, cognition and DEE

327 ALAN affected the proportion of movements at night displayed by birds. Specifically, white
 328 light (estimate = 0.11, SE = 0.03) induced more movement compared to darkness (estimate =
 329 0.04, SE = 0.01, p = 0.02), whereas no difference was found between green light (estimate =
 330 0.09, SE = 0.04) and other treatments (Fig. 2A and Table S2).). However, ALAN had no effect
 331 on the change in levels of blood oxalate (Fig. 2B and Table S2). Similarly, cognition was not
 332 affected by ALAN (Fig. 2C and Table S2). Only the type of task had an effect on the total
 333 number of trials for task completion (p < .001), because in memory tasks birds were quicker
 334 (estimate = 3.3, SE = 0.46), compared to learning tasks (estimate = 6.6, SE = 0.58), independent
 335 of treatment or origin (p>0.1 in both cases). DEE was significantly affected by treatment (p =

336 0.002). Post-hoc tests revealed that birds that were exposed to ALAN, regardless of their origin
 337 and the light spectrum, had a higher DEE compared to the dark control group (DW, $p = 0.003$;
 338 DG, $p = 0.011$, WG, $p=0.87$) (Fig. 2D and Table S2).



339

340 **Figure 2.** Response of great tits to the presence of artificial light at night and different light
 341 spectra. A. Proportion of minutes at night that birds spent without head tucked under feathers.
 342 B. Oxalix acid calculated as the change in levels between baseline measurement and each
 343 treatment period. C. Total number of trials until task completion during the cognition tests. D.
 344 Daily energy expenditure of birds. Bars and error bars represent raw data as means \pm SEM.

345

346 Discussion

347 *(a) Great tits prefer to sleep under green ALAN*

348 ALAN can have detrimental effects on birds, such as altering susceptibility to infection [39],
 349 increasing stress [30] and inhibiting body mass gain [14]. Moreover, previous studies on free-
 350 living great tits and European blackbirds showed that birds might avoid illuminated areas at
 351 night [11,21] possibly in an attempt to evade the adverse effects of nocturnal light. Therefore,
 352 we hypothesized that birds would prefer to roost in darkness. Contrary to our expectations, in
 353 the choice experiment, birds had a clear preference for roosting under light instead of darkness.

354 In particular, they chose to sleep under green light more often when the alternative choice was
355 white light or darkness, and white light was also (slightly) preferred over darkness.

356 While we do not know the exact mechanism behind the choice of roosting under light
357 versus darkness, we suggest that when light intensity is dimmed enough, birds prefer to roost
358 under light to extend their days and possibly increase foraging time and extra-pair mate
359 attraction (assuming that such preference does not vary seasonally, since these experiments
360 were run in autumn). Indeed, in birds extension of activity into the night under ALAN has been
361 associated with increased extra-pair paternity gain [10] and food intake [40]. The benefits of
362 increase foraging at night might be particularly beneficial in winter, when the energetic costs
363 of thermoregulation during cold nights might impose strong selection on the ability of birds to
364 acquire sufficient food to survive the night. However, we also stress that our birds were held
365 in captivity with constant warm temperature and ad libitum food. Future captive studies could
366 deprive birds of food at night to test whether birds would still extend their activity into the
367 night under ALAN.

368

369 *(b) The physiological and behavioural consequences of sleeping under ALAN*

370 The preference for birds to sleep under artificial light raises the question of whether ALAN
371 exposure has any real negative effect on health, cognition and ultimately fitness. In the follow
372 up of the choice experiment, the forced light exposure experiment, both light treatments had a
373 strong effect on activity patterns, but white light more so compared to green light. In particular,
374 the largest differences were seen in the activity onset of birds, where birds started their day
375 around 30 minutes earlier under white light compared to green light. Conversely, birds under
376 dark nights confined their activity during the daylight hours. A similar experiment with blue
377 tits showed the same pattern, with white light having a more severe effect on nocturnal activity
378 compared to green light [31]. Interestingly, many of these effects of activity patterns plateaued

379 or even reversed after a few days of exposure (Fig. S2), possibly suggesting habituation to
380 light. Future studies should directly test this hypothesis.

381 Parallel to these strong changes in activity patterns, birds under green and white light
382 showed elevated levels of DEE. Our findings support the idea that an increase in locomotor
383 activity could lead to higher levels of DEE. The increase in DEE under ALAN found in our
384 experiment contradicts what was found in a recent study of Welbers et al. [20], where a lower
385 DEE was observed under experimental green and white lights installed in forest areas.
386 However, this study was conducted on free-living birds, and it was suggested that the decrease
387 in DEE might be related to other ecological factors, such as the attraction of insects to light and
388 thus increase in food availability in illuminated areas. Such factors were missing in our
389 laboratory experiment, which might explain the discrepancy in the data from the field. Field
390 data might be more biologically relevant. However, great tits are more often exposed to light
391 at night in urban areas, where availability of preferred insect preys is usually scarce [41,42].
392 Indeed, the only previous study which measured DEE in urban and rural great tits found energy
393 expenditure to be higher in urban individuals [43].

394 Despite the effects of ALAN on nocturnal activity and energy expenditure, no clear
395 impact on sleep disruption was found, as measured by the plasma levels of oxalic acid. There
396 could be several reasons for this. One potential explanation is that oxalic acid is not a valid
397 biomarker for sleep debt in birds, as studies so far are contradictory. In fact, in a previous
398 experimental study in the field, birds living in forest areas that were artificially illuminated did
399 show more nocturnal restlessness and a reduction in oxalic acid over time [24]. A more recent
400 study showed that levels of oxalic acid increased in great tit nestlings exposed to ALAN in
401 their nest boxes [44]. However, in this study it was also noted that sleep patterns differed
402 between developing birds and adults, which may reflect age-specific differences in sleep loss
403 in response to ALAN and thus, changes in levels oxalic acid. An alternative explanation is that

404 our experimental treatment of 1.5 lux of ALAN was not sufficiently strong to cause sleep
405 disruption and ultimately alter cognitive responses. As mentioned in the introduction, an
406 intensity of light of 1.5 lux is within the range of what wild birds can be exposed to in light
407 polluted areas. However, artificial light levels measured underneath street lamps can be as high
408 as 20 lux, and on average between 5 and 10 lux [3,11]. Thus, 1.5 lux might simply represent a
409 level of light that birds can tolerate without suffering sleep disruption and an associated
410 reduction in oxalic acid level.

411 We hypothesized that ALAN would have an effect on cognition as cognitive abilities
412 in birds, like learning and memory, can be affected by sleep quality [45], and nocturnal
413 illumination can lead to restlessness [14,24]. Contrary to our expectations, we did not find any
414 effects of ALAN on cognition, which might be due to several reasons. First, birds in our
415 experiment might not have experienced sleep disruption (see above). Previous studies showed
416 that very high levels of light at night can have detrimental effects on cognition of birds. For
417 instance, birds exposed to constant daylight for the whole 24h showed a decrease in neuronal
418 activity in brain regions associated with cognition and a decline in cognitive functions [46].
419 However, our experimental manipulation was closer to a natural situation compared to these
420 previous studies, as essentially birds were still exposed to LD cycles with only dim light at
421 night. Thus, as mentioned above, the birds in our experiment might not have experienced the
422 same degree of circadian sleep disruption under dim light at night and consequently cognitive
423 responses were not altered.

424

425 *(c) Urban and forest great tits respond differently to ALAN*

426 Urban and forest birds respond differently to ALAN in many features of their activity patterns.
427 ALAN, and in particular white light, was consistently more disruptive on the activity patterns
428 of forest birds compared to urban conspecifics. In the forced light exposure experiment, while

429 the amount of nocturnal activity and total activity was similar for both urban and forest birds
430 under green light, it was higher for forest birds under white light. Thus, our results suggest that
431 forest birds are more sensitive to nocturnal lighting, and in particular to white light, than urban
432 birds. It has been proposed that prolonged exposure to anthropogenic factors, including ALAN,
433 should lead to acclimation or even adaptation, resulting in habitat-specific differences in
434 behaviour and physiology between populations inhabiting urban and forest areas [47,48].
435 However, such differences might depend on the specific biological function considered, and
436 also on the species. Indeed, when exposed to ALAN, blackbirds from city areas showed a
437 stronger reproductive response [11], but no difference in daily activity pattern [15], compared
438 to forest conspecifics. In a common-garden experiment, urban blackbirds also showed lower
439 responsiveness of the stress axis compared to forest conspecifics [48]. The increase in night
440 activity and total activity for forest birds under white light compared to green light, and the
441 lack of differences between the two light treatments for urban birds, supports the idea that white
442 lights could possibly have stronger effects on activity patterns of naïve animals. However, in
443 our experiment origin was assigned to birds depending on the location in which the birds were
444 caught. We had no knowledge on the previous experiences of the birds, and as such we cannot
445 directly relate our outcomes to previous light exposure.

446

447 *(d) Light spectra matter*

448 The different spectra we used clearly had an effect on light preference and activity patterns.
449 Birds preferred to sleep under green light, and white light had the strongest effects on activity
450 levels. We deployed green and white lights with the same measured illuminance, that is, lux
451 levels. However, lux is a unit of measure that is calibrated to the photo-sensitivity of the human
452 eye. We used these human-based light measurements in lux because these will be the real
453 currency when city councils install new lights, regardless of the action spectra of wild animals.

454 We recognized that the avian action spectrum is different from that of humans [5]. However,
455 the spectral characteristics of the visual system are a limited predictor of how intense birds
456 perceive light. For instance, a study by Prayitno and Philips [49] showed that the difference in
457 perceived colour-dependent light intensity (in a discrimination test) can be difficult to predict
458 from the known spectral sensitivity of the eye. Moreover, the circadian system of birds is
459 complex and relies upon the action of several types of photoreceptors located in different areas,
460 including the retina, the pineal gland and the hypothalamus [5]. Our understanding of which
461 set of photoreceptors may be more affected by dim artificial light at night, and how in turn they
462 might affect circadian behaviour and physiology, is currently scarce. This limits our ability to
463 understand the mechanisms by which light pollution affects the circadian system of birds and
464 other animals. Future studies should look at filling this gap.

465

466 *(e) Conclusion*

467 We provide the first and only evidence that a wild bird species prefers to roost under light
468 instead of darkness when given the choice in the lab. We proposed the idea that birds may
469 actively select to roost under light at night when this is sufficiently dim not to disrupt their
470 sleep, as this offers the opportunity for increased foraging at night, which has been shown in
471 other species [40,50]. From our camera recordings we could detect nocturnal foraging in some
472 birds, although this was difficult to quantify. Moreover, birds clearly preferred to roost under
473 green light and light levels of 1.5 lux did not likely result in sleep disruption and cognitive
474 impairment. Thus, negative behavioural and physiological effects of ALAN might be observed
475 only under intensities higher than 1.5 lux. These results suggest that reducing the intensity of
476 light pollution as well as tuning the spectrum towards long wavelengths may considerably
477 reduce its impact. Such simple, clear guidelines should be taken into considerations when
478 installing new artificial illumination.

479

480 **Acknowledgements.** We wish to thank all the people who helped us catching birds in the field:
481 Robin Heinen, Martijn van der Sluijs, Peter Alblas. We are also grateful to Coretta Jongeling,
482 Ruben de Wit, Franca Kropman, Marylou Aaldering for providing outstanding care to all the
483 birds in this experiment. Jeroen Laurens and Gilles Wijlhuizen provided technical assistance
484 for the installation of the cages, the light system and the activity recorders. Kees van Oers
485 helped designing the cognitive tests and lent us the necessary equipment.

486

487 **References**

- 488 1. Dominoni D, Borniger J, Nelson R. 2016 Light at night, clocks and health: from
489 humans to wild organisms. *Biol. Lett.* **12**, 20160015. (doi:10.1098/rsbl.2016.0015)
- 490 2. Sanders D, Gaston KJ. 2018 How ecological communities respond to artificial light at
491 night. *J. Exp. Zool. Part A Ecol. Integr. Physiol.* (doi:10.1002/jez.2157)
- 492 3. Longcore T, Rich C. 2004 Ecological light pollution. *Front. Ecol. Environ.* **2**, 191–
493 198. (doi:10.1890/1540-9295(2004)002[0191:ELP]2.0.CO;2)
- 494 4. Van Doren BM, Horton KG, Dokter AM, Klinck H, Elbin SB, Farnsworth A. 2017
495 High-intensity urban light installation dramatically alters nocturnal bird migration.
496 *Proc. Natl. Acad. Sci.* , 201708574. (doi:10.1073/PNAS.1708574114)
- 497 5. Cassone VM. 2014 Avian circadian organization: A chorus of clocks. *Front.*
498 *Neuroendocrinol.* **35**, 76–88. (doi:10.1016/j.yfrne.2013.10.002)
- 499 6. Dominoni DM, Partecke J. 2015 Does light pollution alter daylength? A test using
500 light-loggers on free-ranging European blackbirds (*Turdus merula*). *Philos. Trans. R.*
501 *Soc. B Biol. Sci.* **370**, 20140118.
- 502 7. de Jong M, Jeninga L, Ouyang JQ, van Oers K, Spoelstra K, Visser ME. 2016 Dose-
503 dependent responses of avian daily rhythms to artificial light at night. *Physiol. Behav.*

- 504 **155**, 172–179. (doi:10.1016/j.physbeh.2015.12.012)
- 505 8. Dominoni D, de Jong M, Bellingham M, O’Shaughnessy P, van Oers K, Robinson J,
506 Smith B, Visser ME, Helm B. 2018 Dose-response effects of light at night on the
507 reproductive physiology of great tits (*Parus major*): Integrating morphological analyses
508 with candidate gene expression. *J. Exp. Zool. Part A Ecol. Integr. Physiol.* **2018**, 1–15.
509 (doi:10.1002/jez.2214)
- 510 9. Kumar J, Malik S, Bhardwaj SK, Rani S. 2018 Bright light at night alters the
511 perception of daylength in Indian weaver bird (*Ploceus philippinus*). *J. Exp. Zool.*
512 *Part A Ecol. Integr. Physiol.* (doi:10.1002/jez.2201)
- 513 10. Kempenaers B, Borgström P, Loës P, Schlicht E, Valcu M. 2010 Artificial night
514 lighting affects dawn song, extra-pair siring success, and lay date in songbirds. *Curr.*
515 *Biol.* **20**, 1735–1739. (doi:10.1016/j.cub.2010.08.028)
- 516 11. Dominoni D, Quetting M, Partecke J. 2013 Artificial light at night advances avian
517 reproductive physiology. *Proc. R. Soc. B Biol. Sci.* **280**, 20123017.
518 (doi:10.1098/rspb.2012.3017)
- 519 12. Nordt A, Klenke R. 2013 Sleepless in town - Drivers of the temporal shift in dawn
520 song in urban European blackbirds. *PLoS One* **8**, 1–10.
521 (doi:10.1371/journal.pone.0071476)
- 522 13. Da Silva A, Samplonius J, Schlicht E, Valcu M, Kempenaers B. 2014 Artificial night
523 lighting rather than traffic noise affects the daily timing of dawn and dusk singing in
524 common European songbirds. *Behav. Ecol.* **25**, 1037–1047.
525 (doi:10.1093/beheco/aru103)
- 526 14. Raap T, Pinxten R, Eens M. 2015 Light pollution disrupts sleep in free-living animals.
527 *Sci. Rep.* **5**, 13557. (doi:10.1038/srep13557)
- 528 15. Dominoni D, Goymann W, Helm B, Partecke J. 2013 Urban-like night illumination

- 529 reduces melatonin release in European blackbirds (*Turdus merula*): implications of
530 city life for biological time-keeping of songbirds. *Front. Zool.* **10**, 60.
531 (doi:10.1186/1742-9994-10-60)
- 532 16. Aulsebrook AE, Jones TM, Mulder RA, Lesku JA. 2018 Impacts of artificial light at
533 night on sleep: A review and prospectus. *J. Exp. Zool. Part A Ecol. Integr. Physiol.*
534 (doi:10.1002/jez.2189)
- 535 17. Ouyang JQ, Davies S, Dominoni D. 2018 Hormonally mediated effects of artificial
536 light at night on behavior and fitness: linking endocrine mechanisms with function. *J.*
537 *Exp. Biol.* **221**, jeb156893. (doi:10.1242/jeb.156893)
- 538 18. Careau V, Killen SS, Metcalfe NB. 2015 Adding Fuel to the ‘Fire of Life’: Energy
539 Budgets across Levels of Variation in Ectotherms and Endotherms. In *Integrative*
540 *Organismal Biology*, pp. 219–233. (doi:10.1002/9781118398814.ch14)
- 541 19. Nagy KA. 2005 Field metabolic rate and body size. *J. Exp. Biol.* **208**, 1621–1625.
542 (doi:10.1242/jeb.01553)
- 543 20. Welbers AAMH, van Dis NE, Kolvoort AM, Ouyang J, Visser ME, Spoelstra K,
544 Dominoni DM. 2017 Artificial Light at Night Reduces Daily Energy Expenditure in
545 Breeding Great Tits (*Parus major*). *Front. Ecol. Evol.* **5**, 1–10.
546 (doi:10.3389/fevo.2017.00055)
- 547 21. de Jong M, Ouyang JQ, van Grunsven RHA, Visser ME, Spoelstra K. 2016 Do Wild
548 Great Tits Avoid Exposure to Light at Night? *PLoS One* **11**, e0157357.
549 (doi:10.1371/journal.pone.0157357)
- 550 22. Cho YM, Ryu SH, Lee BR, Kim KH, Lee E, Choi J. 2015 Effects of artificial light at
551 night on human health: A literature review of observational and experimental studies
552 applied to exposure assessment. *Chronobiol. Int.* **32**, 1294–1310.
553 (doi:10.3109/07420528.2015.1073158)

- 554 23. Weljie AM, Meerlo P, Goel N, Sengupta A, Kayser MS, Abel T, Birnbaum MJ,
555 Dinges DF, Sehgal A. 2015 Oxalic acid and diacylglycerol 36:3 are cross-species
556 markers of sleep debt. *Proc. Natl. Acad. Sci.* **112**, 2569–2574.
557 (doi:10.1073/pnas.1417432112)
- 558 24. Ouyang JQ, de Jong M, van Grunsven RHA, Matson KD, Hausmann MF, Meerlo P,
559 Visser ME, Spoelstra K. 2017 Restless roosts – light pollution affects behavior, sleep
560 and physiology in a free-living songbird. *Glob. Chang. Biol.* , 1–8.
- 561 25. Vorster AP, Born J. 2015 Sleep and memory in mammals, birds and invertebrates.
562 *Neurosci. Biobehav. Rev.* **50**, 103–119. (doi:10.1016/j.neubiorev.2014.09.020)
- 563 26. Brodin A, Urhan AU. 2014 Interspecific observational memory in a non-caching Parus
564 species, the great tit Parus major. *Behav. Ecol. Sociobiol.* **68**, 649–656.
565 (doi:10.1007/s00265-013-1679-2)
- 566 27. Jha NA, Kumar V. 2017 Effect of no-night light environment on behaviour, learning
567 performance and personality in zebra finches. *Anim. Behav.* **132**, 29–47.
568 (doi:10.1016/j.anbehav.2017.07.017)
- 569 28. Kyba CCM *et al.* 2017 Artificially lit surface of Earth at night increasing in radiance
570 and extent. *Sci. Adv.* **3**, e1701528. (doi:10.1126/sciadv.1701528)
- 571 29. de Jong M, Ouyang JQ, Da Silva A, van Grunsven RHA, Kempnaers B, Visser ME,
572 Spoelstra K. 2015 Effects of nocturnal illumination on life-history decisions and
573 fitness in two wild songbird species. *Philos. Trans. R. Soc. B Biol. Sci.* **370**,
574 20140128–20140128. (doi:10.1098/rstb.2014.0128)
- 575 30. Ouyang JQ, de Jong M, Hau M, Visser ME, Grunsven RHA Van, Spoelstra K. 2015
576 Stressful colours: corticosterone concentrations in a free-living songbird vary with the
577 spectral composition of experimental illumination. *Biol. Lett.* **11**, 20150517.
- 578 31. de Jong M, Caro SP, Gienapp P, Spoelstra K, Visser ME. 2017 Early Birds by Light at

- 579 Night: Effects of Light Color and Intensity on Daily Activity Patterns in Blue Tits. *J.*
580 *Biol. Rhythms* **32**, 323–333. (doi:10.1177/0748730417719168)
- 581 32. Dawkins MS. 1990 From an animal's point of view: Motivation, fitness, and animal
582 welfare. *Behav. Brain Sci.* **13**, 1–9. (doi:10.1017/S0140525X00077104)
- 583 33. Riber AB. 2015 Effects of color of light on preferences, performance, and welfare in
584 broilers. *Poult. Sci.* **94**, 1767–1775. (doi:10.3382/ps/pev174)
- 585 34. Sun J, Raap T, Pinxten R, Eens M. 2017 Artificial light at night affects sleep behaviour
586 differently in two closely related songbird species. *Environ. Pollut.* **231**, 882–889.
587 (doi:10.1016/j.envpol.2017.08.098)
- 588 35. Gaston KJ, Davies TW, Bennie J, Hopkins J. 2012 REVIEW: Reducing the ecological
589 consequences of night-time light pollution: options and developments. *J. Appl. Ecol.*
590 **49**, 1256–1266. (doi:10.1111/j.1365-2664.2012.02212.x)
- 591 36. Szymczak JT, Helb HW, Kaiser W. 1993 Electrophysiological and behavioral
592 correlates of sleep in the blackbird (*Turdus merula*). *Physiol. Behav.* **53**, 1201–10.
593 (doi:Doi: 10.1016/0031-9384(93)90380-x)
- 594 37. Mitchell GW, Guglielmo CG, Hobson KA. 2015 Measurement of Whole-Body CO₂
595 Production in Birds Using Real-Time Laser-Derived Measurements of Hydrogen (δ^2
596 H) and Oxygen (δ^{18} O) Isotope Concentrations in Water Vapor from Breath. *Physiol.*
597 *Biochem. Zool.* **88**, 599–606. (doi:10.1086/683013)
- 598 38. Titulaer M, van Oers K, Naguib M. 2012 Personality affects learning performance in
599 difficult tasks in a sex-dependent way. *Anim. Behav.* **83**, 723–730.
600 (doi:10.1016/j.anbehav.2011.12.020)
- 601 39. Kernbach ME, Miller JM, Hall RJ, Unnasch TR, Burkett-Cadena ND, Martin LB.
602 2018 Light Pollution Increases West Nile Virus Competence in a Ubiquitous Passerine
603 Reservoir Species. *bioRxiv*, 269209. (doi:10.1101/269209)

- 604 40. Russ A, Ruger A, Klenke R. 2015 Seize the night: European Blackbirds (*Turdus*
605 *merula*) extend their foraging activity under artificial illumination. *J. Ornithol.* **156**,
606 123–131. (doi:10.1007/s10336-014-1105-1)
- 607 41. Pollock CJ, Capilla-Lasheras P, McGill RAR, Helm B, Dominoni DM. 2017
608 Integrated behavioural and stable isotope data reveal altered diet linked to low
609 breeding success in urban-dwelling blue tits (*Cyanistes caeruleus*). *Sci. Rep.* **7**, 5014.
610 (doi:10.1038/s41598-017-04575-y)
- 611 42. Seress G, Hammer T, Bokony V, Vincze E, Preiszner B, Pipoly I, Sinkovics C, Evans
612 KL, Liker A. 2018 Impact of urbanization on abundance and phenology of caterpillars
613 and consequences for breeding in an insectivorous bird. *Ecol. Appl.* **28**, 1143–1156.
614 (doi:10.1002/eap.1730)
- 615 43. Hinsley SA, Hill RA, Bellamy PE, Harrison NM, Speakman JR, Wilson AK, Ferns
616 PN. 2008 Effects of structural and functional habitat gaps on breeding woodland birds:
617 Working harder for less. *Landsc. Ecol.* **23**, 615–626. (doi:10.1007/s10980-008-9225-8)
- 618 44. Raap T, Pinxten R, Eens M. 2018 Artificial light at night causes an unexpected
619 increase in oxalate in developing male songbirds. *Conserv. Physiol.* **6**.
620 (doi:10.1093/conphys/coy005)
- 621 45. Rattenborg NC, Martinez-Gonzalez D, Lesku JA. 2009 Avian sleep homeostasis:
622 Convergent evolution of complex brains, cognition and sleep functions in mammals
623 and birds. *Neurosci. Biobehav. Rev.* **33**, 253–270.
624 (doi:10.1016/j.neubiorev.2008.08.010)
- 625 46. Taufique SKKT, Kumar V. 2016 Differential activation and tyrosine hydroxylase
626 distribution in the hippocampal, pallial and midbrain brain regions in response to
627 cognitive performance in Indian house crows exposed to abrupt light environment.
628 *Behav. Brain Res.* **314**, 21–29. (doi:10.1016/j.bbr.2016.07.046)

- 629 47. Bonier F, Martin PR, Wingfield JC. 2007 Urban birds have broader environmental
630 tolerance. *Biol. Lett.* **3**, 670–673. (doi:10.1098/rsbl.2007.0349)
- 631 48. Partecke J, Schwabl I, Gwinner E. 2006 Stress and the city: urbanization and its effects
632 on the stress physiology in European blackbirds. *Ecology* **87**, 1945–52.
633 (doi:10.1890/0012-9658(2006)87[1945:SATCUA]2.0.CO;2)
- 634 49. Prayitno DS, Phillips CJC. 1997 Equating the perceived intensity of coloured lights to
635 hens. *Br. Poult. Sci.* **38**, 136–141. (doi:10.1080/00071669708417958)
- 636 50. Stracey CM, Wynn B, Robinson SK. 2014 Light Pollution Allows the Northern
637 Mockingbird (*Mimus polyglottos*) to Feed Nestlings After Dark. *Wilson J. Ornithol.*
638 **126**, 366–369. (doi:10.1676/13-107.1)
- 639

