

This item is the archived peer-reviewed author-version of:

Cavities shield birds from effects of artificial light at night on sleep

Reference:

Raap Thomas, Pinxten Rianne, Eens Marcel.- Cavities shield birds from effects of artificial light at night on sleep
Journal of experimental zoology: part A : ecological and integrative physiology - ISSN 2471-5646 - 329:8-9(2018), p. 449-456
Full text (Publisher's DOI): <https://doi.org/10.1002/JEZ.2174>
To cite this reference: <https://hdl.handle.net/10067/1539830151162165141>

1 **Cavities shield birds from effects of artificial light at night on sleep**

2

3

4

5 Thomas Raap ^{a*}

6 Rianne Pinxten ^{a, b}

7 Marcel Eens ^a

8

9 ^a Department of Biology, Behavioural Ecology and Ecophysiology Group, University of
10 Antwerp, Wilrijk, Belgium

11

12 ^b Faculty of Social Sciences, Antwerp School of Education, University of Antwerp, Antwerp,
13 Belgium

14

15 * Corresponding author: thomas.raap@uantwerpen.be

16

17 Abbreviated title: Cavities shield birds from ALAN

18 **Abstract**

19 Light pollution is an ever increasing worldwide problem disrupting animal behaviour.
20 Artificial light at night (ALAN) has been shown to affect sleep in wild birds. Even cavity-
21 nesting bird species may be affected when sleeping inside their cavity. Correlational studies
22 suggest that light from outside the cavity/nest box, for example from street lights, may affect
23 sleep. We used an experimental design to study to what extent nest boxes shield animals from
24 effects of ALAN on sleep. We recorded individual sleep behaviour of free-living great tits
25 (*Parus major*) that were roosting in dark nest boxes and exposed their nest box entrance to
26 ALAN the following night (1.6 lux white LED light; a similar light intensity as was found at
27 nest boxes near street lights). Their behaviour was compared to that of control birds sleeping in
28 dark nest boxes on both nights.

29 Our experimental treatment did not affect sleep behaviour. Sleep behaviour of birds in
30 the control group did not differ from that of individuals in the light treated group. Our results
31 suggest that during winter cavities shield birds from some effects of ALAN. Furthermore, given
32 that effects of ALAN and exposure to artificial light are species-, sex- and season-dependent, it
33 is important that studies using wild animals quantify individual exposure to light pollution, and
34 be cautious in the interpretation and generalisation of the effects, or lack thereof, from light
35 pollution. Rigorous studies are necessary to examine individual light exposure and its
36 consequences in cavity- and open-nesting birds.

37

38 **Keywords**

39 artificial light at night; light pollution; model species; sleep behaviour; urbanization

40 **Introduction**

41 Light pollution or artificial light at night (ALAN) is an increasing worldwide
42 environmental alteration (Falchi et al., 2016) and we are just beginning to explore the multitude
43 of its effects. Light pollution disrupts natural light cycles and potentially poses an important
44 threat for wildlife, biodiversity and humans (Duffy, Bennie, Duran, & Gaston, 2015; Gaston,
45 Bennie, Davies, & Hopkins, 2013; Hölker, Wolter, Perkin, & Tockner, 2010; Kyba & Hölker,
46 2013; Navara & Nelson, 2007; Rich & Longcore, 2005) since it results in a wide range of
47 physiological and behavioural responses (see e.g. Da Silva & Kempnaers, 2017; Dominoni,
48 Quetting, & Partecke, 2013). For example, in two cavity-nesting songbird species, blue tits
49 (*Cyanistes caeruleus*) and great tits (*Parus major*), sleep behaviour was disrupted by
50 experimental light inside the nest box (Raap, Pinxten, & Eens, 2015, 2016c; 2017c; Sun, Raap,
51 Pinxten, & Eens, 2017). Sleep is an important animal behaviour with multiple possible
52 functions, enabling animals to recover from daily stress (Siegel, 2009; Weljie et al., 2015), to
53 consolidate memory and to conserve energy (Gobes, Zandbergen, & Bolhuis, 2010; Roth II,
54 Rattenborg, & Pravosudov, 2010; Vorster & Born, 2015).

55 There are several indications why ambient light pollution could be expected to affect
56 sleep behaviour of birds inside cavities/ nest boxes. First, blue and great tits sleeping in nest
57 boxes which were exposed to more (natural) light outside the nest box had an earlier awakening
58 time and leaving time (Steinmeyer, Schielzeth, Mueller, & Kempnaers, 2010; Stuber,
59 Dingemanse, Kempnaers, & Mueller, 2015a). However, due to the correlative nature of these
60 studies confounding effects (e.g. noise) cannot be excluded. Furthermore effects of ALAN were
61 not examined. Second, light pollution may affect sleep as it allows some bird species to forage
62 longer for food (Stracey, Wynn, & Robinson, 2014) which can come at the cost of reduced
63 sleep. There are, however, also indications that suggest that nest boxes may shield animals from
64 direct effects of ALAN. Experimental ALAN inside a nest box affected nestling physiology

65 (Raap, Casasole, Pinxten, & Eens, 2016b), but ambient light pollution at the nest box was
66 unrelated to nestling physiology (Raap et al., 2017a). Whether ambient light pollution leads to
67 altered sleep behaviour of birds inside nest boxes (cavities), similar to what has been found in
68 experiments with ALAN inside a nest box using free-living blue and great tits (Raap et al.,
69 2015, 2016c; 2017c; Sun et al., 2017), needs to be examined.

70 Because correlational relationships between ambient light and the expression of
71 behaviour may reflect indirect effects, we performed an experiment in which we exposed the
72 entrance of great tit nest boxes from the outside to ALAN during the winter period. Dawn
73 singing of one species may affect that of another (Xia et al., 2018) thereby confounding possible
74 effects of light pollution on sleep. However, during winter most species, including great tits, do
75 not yet have a dawn chorus (see e.g. Da Silva, Valcu, & Kempenaers, 2015), excluding the
76 possibility that sleep is affected by dawn song of other species and other great tits. Our
77 experiment more closely resembles light conditions inside cavities experienced in the wild in
78 urban areas, without manipulating daytime behaviours such as extended foraging. We used an
79 outside light source to produce 1.6 lux at the nest box entrance (white LED light). There are
80 several reasons why this treatment could affect sleep behaviour. First, experimental ALAN
81 inside the nest box disrupts sleep behaviour of great tits (Raap et al., 2016c; 2017c). Second,
82 during the night great tits wake up several times per hour (Stuber et al., 2015a) and when a bird
83 sits at the bottom of a nest box it can observe light shining in through the entrance, which could
84 subsequently affect its behaviour. Finally, very low levels of ALAN (0.05 lux) have been found
85 to affect activity onset and offset in great tits in the laboratory (de Jong et al., 2016). However,
86 nestling physiology was unaffected by ambient light pollution (Casasole et al., 2017; Raap et
87 al., 2017a). Therefore, our aim was to test the hypothesis that nest boxes shield birds from the
88 detrimental effects of ambient ALAN on sleep. We recorded individual sleep behaviour of great
89 tits that were roosting in dark nest boxes and exposed their nest box from the outside to ALAN

90 the following night. Their behaviour was compared to that of control birds sleeping in dark nest
91 boxes on both nights. We expected effects in the morning, especially on awakening time (last
92 time the bird was asleep) and leaving time (when the bird leaves the nest box), as natural light
93 in the morning has been shown to relate to these parameters in both blue and great tits
94 (Steinmeyer et al., 2010; Stuber et al., 2015a). Furthermore, during winter our previous
95 experiments with ALAN inside nest boxes also showed most effects to occur during the
96 morning (Raap et al., 2015; 2017c). We used a light intensity for our experimental treatment
97 that was similar to intensities measured at nest boxes located near street lights ($\leq 16\text{m}$; average
98 $1.6 \pm \text{SE } 0.6 \text{ lux}$, $N = 16$; Casasole et al., 2017; Raap et al., 2017a), with street lights themselves
99 often having intensities of around 10-40 lux (Gaston, Davies, Nedelec, & Holt, 2017). The
100 intensity that we used may therefore be experienced by animals near street lights. Getting a
101 better understanding of the effects of light from outside the nest box is highly relevant as it
102 provides insights into the effects of light pollution caused by street lights.

103 **Methods**

104 *Study area and general procedures*

105 Data was collected between February 12th and March 4th 2014 in a resident suburban
106 nest box population of great tits in the surroundings of Wilrijk, Belgium (51°9'44''N,
107 4°24'15''E). This nest box population has been established in 1997 and has been continuously
108 monitored (see e.g. Rivera-Gutierrez, Pinxten, & Eens, 2010, 2012; Thys et al., 2017; Van
109 Duyse, Pinxten, & Eens, 2000; 2005; Vermeulen, Muller, & Eens, 2016). Nest boxes were made
110 out of plywood with a metal ceiling, had outer dimensions of 120 × 155 × 250 mm (width ×
111 depth × height) and an opening of 30 mm ø. During previous winter- and breeding seasons great
112 tits were caught inside nest boxes after which they were sexed and ringed. Since 2011 all birds
113 have been provided with a ring/implant containing a passive integrated transponder (PIT) tag,
114 enabling the individual detection of birds sleeping in nest boxes without physically disturbing
115 them.

116

117 *Experimental procedure*

118 Nest boxes with a maximum nighttime light intensity of 0.3 lux at the entrance hole
119 were selected for this experiment (range: 0.01 - 0.26 lux, average: 0.12 lux; ISO-Tech ILM
120 1335 light meter). After sunset the light intensity inside these nest boxes was ± 0.01 lux, which
121 is the minimum that the light meter can measure. These experimental nest boxes were located
122 far from street lights (>30 m), and experienced a natural light regime. Light intensity from street
123 lights quickly declines within several meters to almost dark levels (Gaston et al., 2017; Raap,
124 Pinxten, & Eens, 2017b).

125 A within-individual design was used in which sleep behaviour was observed over two
126 subsequent nights in a control (dark) treatment and a light treatment. Because of the high

127 variability between individuals in sleep behaviour (Raap et al., 2016c), we used a within-
128 individual design which “controls” for this variation (Ruxton & Colegrave, 2010). This design,
129 where an individual acts as its own control, also increases the statistical power (Seltman, 2013).

130 Birds in the light group slept with the flashlight (see “*Light treatment*”) turned off on
131 the first night and turned on during the second night, while in the control group birds were
132 observed over two nights sleeping in a naturally dark situation. Flashlights were turned on when
133 infrared sensitive cameras were installed, at least two hours before sunset (lights were on for
134 about 18 hours in total; see “*Sleep behaviour recordings*”). We performed observations of sleep
135 behaviour in the control and light group simultaneously over a period of 20 days/nights, with
136 not all individuals being observed during the same night. In total we obtained paired data from
137 seven individuals (three females and four males) in the control group and from ten individuals
138 (four females and six males) in the light group. Because we expected smaller differences in
139 sleep behaviour between nights in the control group (Raap et al., 2015) we recorded fewer
140 individuals in this group compared to the light group.

141

142 *Light treatment*

143 Birds were first allowed to sleep in their normal dark situation and a pole with a dummy
144 flashlight was put up at 5 meters from the nest box (at the same time the camera was installed;
145 at the latest two hours before sunset). The following night we replaced the dummy with a similar
146 sized flashlight (white LED, Xtar R30 XML U2) calibrated to produce about 1.6 lux at the nest
147 box entrance. We used white LED as these light types are increasingly used as street lights
148 (Kyba et al., 2017; Schubert & Kim, 2005). Animals in the control group slept in the dark on
149 both nights with a dummy, similar to the flashlight, installed outside. We used a light intensity
150 of 1.6 lux which is lower than the maximum values in our population of nest boxes near street

151 lights (≈ 8 lux at the outside of the nest box opening) but represents a light intensity which can
152 be found for nest boxes (and cavities) exposed to ALAN from street lights (see also Dominoni
153 et al., 2013; Gaston et al., 2013; 2017). While nest boxes close to street lights (8m; not used in
154 this experiment) can experience light intensities as high as 8 lux on the nest box opening, the
155 light intensity inside at the bottom of the nest box is negligible (0.01 lux, $N = 20$).

156

157 *Sleep behaviour recordings*

158 We used the procedure for recording sleep behaviour as previously described by Raap
159 et al. (2015). Nest boxes were checked for presence and identity of sleeping great tits prior to
160 the first recording and during the experiment with a handheld transponder reader (FR-250 RFID
161 Reader, Trovan, Aalten, Netherlands). To record sleeping behaviour we installed infrared
162 sensitive cameras (Pakatak PAK-MIR5, Essex, UK) under the nest box roof-lid, at least two
163 hours before sunset and removed them, at the earliest, two hours after sunrise the next morning.
164 Recordings started after the cameras were installed. Birds were never present inside the nest
165 box during the time of installation.

166

167 *Defining sleep behaviour*

168 As great tits readily sleep in nest boxes, they are an ideal model species to study sleep
169 behaviour (and physiology) in free-living animals and to manipulate the light conditions to
170 which they are exposed to during the night (e.g. Raap et al., 2017c). Unfortunately they are too
171 small to be fitted with modern data loggers, which would otherwise enable recording of their
172 brain activity (necessary for defining sleep). We acknowledge that sleep behaviour remains a
173 proxy for sleep and has its limitations (Aulsebrook, Jones, Rattenborg, Roth II, & Lesku, 2016)

174 but it is difficult to study sleep in the wild (Rattenborg et al., 2017). Nonetheless, sleep
175 behaviour is ecologically relevant as it has been linked to behavioural changes, genetic variation
176 and fitness-related traits (Amo, Caro, & Visser, 2011; Christe, Richner, & Oppliger, 1996;
177 2013; Steinmeyer et al., 2010; 2016; 2015a; Stuber et al., 2014; 2015b; Tripet, Glaser, &
178 Richner, 2002). Previous work in blackbirds (*Turdus merula*) also showed close
179 correspondence between behaviourally observed and electrophysiological measured sleep
180 (Szymczak, Helb, & Kaiser, 1993).

181 Similar to other relevant work on great and blue tits, we thus defined sleep entirely by
182 using sleep behaviour (Raap et al., 2015, 2016c; 2013; Steinmeyer et al., 2010; 2015a; Stuber
183 et al., 2014; Sun et al., 2017). When a bird showed the classical sleep position (beak pointing
184 backwards and tucked under the scapulars), it was considered to be sleeping (Amlaner & Ball,
185 1983). However, in rare cases, individuals sat quietly for some time with the head pointing
186 forwards or not completely tucked under the scapular. These periods were defined as awake as
187 they were often followed by the classical sleep position. Sleep of great tits was quantified in
188 detail, as described in earlier studies on great and blue tit sleep behaviour (e.g. Raap et al., 2015;
189 Steinmeyer et al., 2010), using 12 parameters: entry time (min), sleep onset (min), evening
190 latency (min), awakening time (min), leaving time (min), morning latency (min), time on
191 entrance (min), number of times on entrance, sleep proportion, sleep bout length (min), sleep
192 bout/ hour, sleep amount (min). For a detailed description of these 12 parameters and how they
193 were scored, please see Raap et al. (2015) and the supplementary material in Raap et al. (2016c).

194

195 *Data analysis*

196 For all statistical analyses we used R 3.2.2 (R Core Team, 2016). We converted entry
197 time, sleep onset, awakening time and leaving time to times relative to sunset or sunrise
198 (reference data from Antwerp were used; Royal Observatory Belgium).

199 For each sleep parameter a separate linear mixed effect model was constructed (using
200 the lme4 package; Bates et al., 2013). As dependent variable we used the different sleep
201 parameters. The full model was constructed with “Sex”, “Date” (Julian day), “Treatment”
202 (control, light), “Night” (1 or 2) and the interaction “Night:Treatment” as fixed effects, to look
203 at whether the light treatment affected sleep behaviour. We did not take into account a possible
204 sex-dependent effect of our treatment because this is unlikely to be the case (Raap et al., 2017c).
205 Because we used a within-individual design (repeated measures) we included individual
206 identity as a random factor. We tested whether our light treatment affected sleep behaviour by
207 using likelihood ratio tests to compare the full models against the models without the interaction
208 “Night:Treatment”. Generalized linear mixed models were used for “numbers of time on the
209 entrance” (visits on entrance; Poisson distribution) and proportion of time asleep (binomial
210 distribution). We checked normality of dependent variables using histograms (Zuur, Ieno, &
211 Elphick, 2010) and validated models by inspecting residual plots (Zuur et al., 2009). Based on
212 the variation inflation factor there was no multicollinearity.

213

214 **Ethical statements**

215 This study was approved by the ethical committee of the University of Antwerp (ID
216 number 2014-45) and performed in accordance with Belgian and Flemish laws. The Belgian
217 Royal Institute for Natural Sciences provided ringing licenses for all authors and field
218 technicians.

219 **Results**

220 There was no effect of our experimental light, which only exposed the entrance of the
221 nest box to ALAN and not the environment, on any of the sleep parameters, as indicated by
222 non-significant “Night:Treatment” interactions (all $P > 0.255$; Table 1; Figure 1). We obtained
223 estimates and confidence intervals for visualisation purposes which clearly showed that the
224 sleep behaviour of birds in the control group did not change from night one to night two (Figure
225 1). Likewise, the sleep behaviour of animals sleeping in a nest box exposed to our ALAN
226 treatment did not differ between the dark versus illuminated night or from the control group
227 (Figure 1). Birds spent about one minute on the nest box opening (1.1 ± 0.3 minutes; Table 1)
228 and this was not affected by our treatment.

229 Birds slept less as the season progressed (sleep amount, -3.7 ± 0.8 minutes/ day, $F =$
230 23.709 , $P < 0.001$). Males slept less than females (-26.9 ± 8.3 minutes, $F = 10.570$, $P = 0.009$),
231 woke up earlier (5.7 ± 5.8 minutes, $F = 7.266$, $P = 0.012$), left the nest box earlier (-18.9 ± 6.2
232 minutes, $F = 9.216$, $P = 0.005$) and took slightly longer to leave the nest box after waking up
233 (morning latency, 3.7 ± 1.3 minutes, $F = 7.635$, $P = 0.010$).

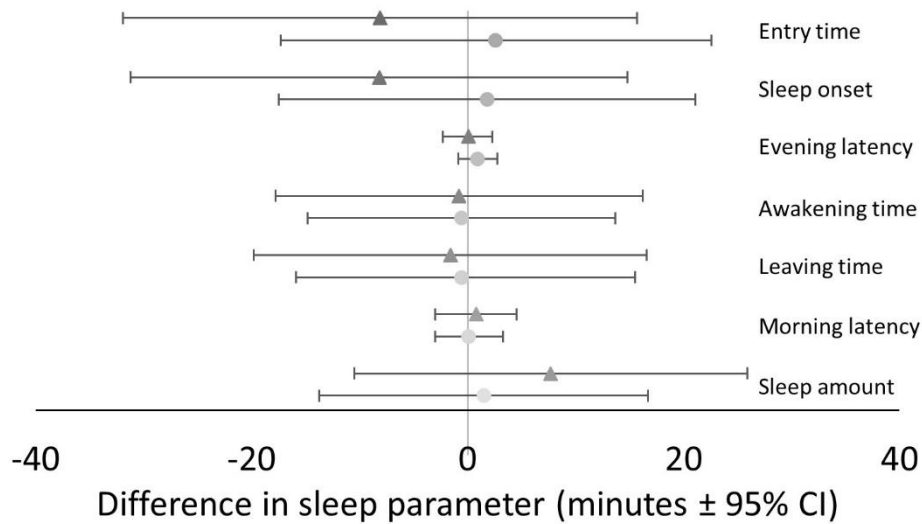
234

235 **Table 1** Results of the mixed effect models on sleep parameters. To correct for changes in day
 236 length, response variables were standardized to civil sunset (entry time, sleep onset) or sunrise
 237 (awakening time and leaving time). LMM models were used with nest identity as random factor
 238 to correct for repeated measurements ($N = 17$). Significant P values are indicated in bold.

	Intercept		Date					Sex					Night:Treatment				
	Estimate	SE	Estimate	SE	DF	<i>F</i>	<i>P</i>	Estimate	SE	DF	<i>F</i>	<i>P</i>	Estimate	SE	DF	<i>F</i>	<i>P</i>
Entry time	44.1	46.2	-0.8	0.8	7.6	0.861	0.382	8.2	8.3	26.3	0.969	0.334	10.7	14.9	21.2	0.521	0.479
Sleep onset	36.2	45.6	-0.7	0.8	7.8	0.718	0.422	7.8	8.1	26.2	0.940	0.341	10.0	14.4	21.4	0.478	0.497
Evening latency	8.2	4.1	-0.1	0.1	7.8	1.191	0.308	0.3	0.8	26.6	0.204	0.655	0.8	1.4	21.3	0.325	0.575
Awakening time	29.5	27.5	-0.8	0.5	28.0	2.384	0.134	-15.7	5.8	28.0	7.266	0.012	0.2	10.8	28.0	0.000	0.984
Leaving time	30.5	29.4	-0.8	0.5	28.0	2.494	0.126	-18.9	6.2	28.0	9.216	0.005	1.0	11.6	28.0	0.007	0.934
Morning latency	-0.5	8.4	0.1	0.2	8.9	0.131	0.726	3.7	1.3	25.9	7.635	0.010	-0.8	2.4	22.4	0.102	0.753
Time on entrance	-3.2	2.0	0.1	0.0	7.8	4.069	0.079	1.3	0.4	8.9	9.466	0.013	-0.6	0.6	15.0	0.975	0.339
Number of times on entrance	0.8	1.3	0.0	0.0	26.0	0.215	0.643	0.5	0.3	26.0	2.864	0.091	-0.1	0.5	26.0	0.023	0.880
Sleep proportion	-2.8	0.7	0.0	0.0	26.0	0.002	0.962	0.1	0.2	26.0	0.855	0.355	-0.1	0.3	26.0	0.052	0.820
Sleep bout length	20.6	8.3	-0.1	0.2	13.0	0.770	0.396	-2.5	1.8	13.0	1.980	0.183	1.0	1.2	15.0	0.749	0.400
Sleep bout/ hour	-0.1	7.5	0.1	0.1	3.4	0.531	0.513	0.8	1.0	2.2	0.558	0.506	-0.6	0.5	15.0	1.402	0.255
Sleep amount	953.6	41.4	-3.7	0.8	8.9	23.709	<0.001	-26.9	8.3	9.8	10.570	0.009	-6.1	11.1	15.0	0.306	0.588

239

240



241

242 **Figure 1** The experimental light treatment, of 1.6 lux at the nest box entrance, did not affect
 243 sleep behaviour. Differences in sleep behaviour between nights for animals in the control group
 244 (triangles) and in the light treated group (circles) are given. We used mixed models with nest
 245 identity as random factor to correct for repeated measurements ($N = 17$). For visual purposes
 246 we extracted effect sizes with 95% confidence intervals, therefore we used Least Squares Means
 247 for post-hoc analyses on all normally distributed sleep parameters (using the lmerTest package;
 248 Kuznetsova et al., 2014).

249

250

251

252

253

254

255

256 **Discussion**

257 We found no evidence that sleep behaviour of free-living great tits was affected by our
258 experimental light, which only exposed the entrance of the nest box to ALAN and not the
259 environment. With our experimental treatment, we wanted to isolate effects of light on sleep
260 behaviour from any other possible confounding effects which may indirectly affect sleep
261 behaviour, such as those through extended foraging behaviour (Stracey et al., 2014). Based on
262 correlational studies showing that great tits that slept in brighter nest boxes woke up earlier
263 (Stuber et al., 2015a) and because experimental ALAN inside the nest box advanced awakening
264 time (Raap et al., 2015; 2017c), we could expect effects. Furthermore very low light intensities
265 (0.05 lux) also caused great tits to advance their activity (de Jong et al., 2016). However, both
266 the timing and duration of sleep behaviour were unaffected. In the following we discuss our
267 results and their possible implications.

268 Although we used a within-individual design, which is powerful to detect changes in
269 behaviour (Seltman, 2013), it might be that our relatively small sample size made it difficult to
270 detect changes in sleep behaviour. From a power analysis it seems that, for example for
271 awakening time, we would need about double the sample size to obtain a 80% power to detect
272 a 20 minute difference in the light treated group. This is an effect size similar to our
273 experimental studies with ALAN inside the nest box (Raap et al., 2017c). We therefore
274 recommend experiments with larger sample sizes to validate our results.

275 The light source (flashlight) in our experimental design was set perpendicular to the nest
276 box opening, which differs from street lights. However, how we exposed the nest box to ALAN
277 is unlikely to explain the lack of effect, as even in nest boxes which are exposed to higher light
278 intensities from street lights (≈ 8 lux on the opening) we measured no light on the bottom of the
279 nest box (pers. obs.). The size of the nest box opening and its relatively high position in the nest

280 box make it very difficult for any light to directly reach the bottom of the nest box, where great
281 tits roost during the winter. However, birds sitting at the bottom of the nest box can observe
282 whether light shines in through the entrance, which could subsequently affect their behaviour.
283 Our experimental treatment lasted only for a single night and therefore we cannot exclude the
284 possibility that a longer light treatment might have elicited effects on sleep behaviour. However,
285 our findings showing no effect of ALAN seem to be in line with a previous study by Titulaer
286 et al. (2012). They used an experimental setup with a light on top of great tit nest boxes during
287 the nestling period for 9 consecutive days (due to nest material great tits will be closer to the
288 nest box opening). They used a white LED light with an intensity of 10 lux at the entrance. In
289 their study they also did not find an effect on activity offset or onset, two behavioural parameters
290 similar to the parameters 'entry and leaving time' that were used in the present study.

291 Effects of light pollution on sleep are likely because the external environment is
292 manipulated, causing changes in dawn song (Kempnaers et al., 2010) or extended foraging
293 (Stracey et al., 2014), rather than direct exposure to ALAN while inside the cavity/ nest box.
294 Sleep behaviour of, for example, great tits might also be affected by the presence of other
295 species that are active earlier in the morning, as dawn song of one species may affect that of
296 another (Xia et al., 2018). Several songbird species, such as robins (*Erithacus rubecula*) and
297 blackbirds, have a naturally earlier (about 20-30 minutes) dawn song than great tits. Exposure
298 to light pollution further advances their dawn song (30-60 minutes or more; Da Silva,
299 Samplonius, Schlicht, Valcu, & Kempnaers, 2014; Kempnaers et al., 2010). The singing
300 behaviour of other species could affect the sleep behaviour of great tits under natural conditions
301 explaining why natural variation in morning light intensity influences leaving time (Stuber et
302 al., 2015a). The study by Stuber et al. (2015a) included data from March when more bird species
303 sing around dawn (Da Silva et al., 2015), which can help in explaining why birds in brighter
304 box locations exited their boxes earlier in the morning in their study. Light pollution is,

305 however, often associated with noise pollution (Halfwerk & Slabbekoorn, 2015) and can also
306 advance dawn song (Fuller, Warren, & Gaston, 2007; Gil, Honarmand, Pascual, Pérez-Mena,
307 & Macías Garcia, 2015) and effects of light and noise pollution are therefore difficult to
308 disentangle. Da Silva et al. (2014) found that light but not noise advanced dawn song in the
309 European robin, the common blackbird, the song thrush (*Turdus philomelos*), the great tit and
310 the blue tit. Arroyo-Solis et al. (2013) on the other hand, found the opposite for the spotless
311 starling (*Sturnus unicolor*) and the house sparrow (*Passer domesticus*). Whether light or noise
312 pollution affects the timing of dawn song may in part be species-dependent. Our treatment did
313 not expose the larger area around the nest box to ALAN thereby isolating effects of light on
314 sleep behaviour from any other possible confounding effects. Our experimental treatment
315 therefore unlikely affected the singing behaviour of other birds. Furthermore, as our experiment
316 was performed during winter, dawn song of most species should still have been very limited at
317 that moment (see e.g. Da Silva et al., 2015).

318 Effects of light pollution on cavity-nesting species are potentially not only species-
319 dependent (Sun et al., 2017) but also sex and season may play an important role and interact
320 with each other. Although our study was done during winter when both male and female great
321 tits roost inside cavities and nest boxes, during the breeding season mainly females sleep inside
322 nest boxes (Hinde, 1952; Kluijver, 1950). Males are therefore possibly exposed to higher levels
323 of light pollution, which could explain results observed on dawn song, a typical male behaviour
324 in great tits (Da Silva & Kempenaers, 2017; Da Silva et al., 2014; 2015; 2016). In our current
325 experimental study performed during the winter period we did not find any effects of our light
326 treatment on sleep behaviour of male and female great tits. Previously we also found no effects
327 of ambient light pollution (caused by street lights) on great tit nestlings' physiology (Casasole
328 et al., 2017; Raap et al., 2017a) while several important indicators of immunity, health, and
329 physiological condition were affected in nestlings experimentally exposed to two nights of

330 ALAN inside the nest box. ALAN caused elevated haptoglobin levels, decreased nitric oxide
331 levels, and nestlings did no longer gain any body mass (Raap et al., 2016a; 2016b) and in male
332 nestlings oxalate, a cross-species biomarker for sleep debt (Weljie et al., 2015), seemed to be
333 affected (Raap et al., 2018). Thus during winter, nest boxes/cavities may provide shielding for
334 both sexes while during the breeding season exposure to light pollution is likely sex-dependent
335 for adults. Exposure to light pollution is not only highly variable for cavity-nesting species light
336 but also for open-nesting species. For example, Dominoni et al. (2013) showed that urban
337 blackbirds were exposed to a large range of light intensities. While city street lights had a light
338 intensity of around 6 lux, males were exposed to a mean intensity of 0.3 and maximum of about
339 2.5 lux. Furthermore, not only may exposure to light pollution vary greatly among individuals
340 but also from one night to another (Dominoni, Carmona-Wagner, Hofmann, Kranstauber, &
341 Partecke, 2014). It is therefore important that studies using wild animals quantify individual
342 exposure to light pollution (Raap et al., 2017b), and be cautious in the interpretation and
343 generalisation of the effects, or lack thereof, from light pollution.

344 We conclude that our light treatment, in which we experimentally exposed the entrance
345 of the nest box to ALAN (1.6 lux white LED), independent of the rest of the environment, had
346 no effect on great tit sleep, while direct exposure to ALAN does disrupt sleep (Raap et al., 2015;
347 2017c). We therefore hypothesize that artificial light at night resulting from street lights may
348 have a limited direct effect on sleep of birds inside cavities during winter. Light pollution is a
349 growing problem which disrupts the timing of a wide variety of animals (Gaston et al., 2017)
350 but under certain circumstances animals might, due to limited exposure, not suffer from direct
351 effects. Future studies should examine individual light exposure and its consequences for cavity
352 and open-nesting birds throughout different seasons.

353 **Acknowledgements**

354 We thank Geert Eens, Peter Scheys and Griet Van Schoote for important support during
355 fieldwork. We are also very thankful to Benjamin Van der Jeught for help in analysing the sleep
356 behaviour and to Nina Dehnhard, Andrea Grunst and Melissa Grunst for valuable feedback.

357 This study was made possible through financial support from the University of Antwerp (to TR,
358 RP, and ME) and from the FWO Flanders through a PhD fellowship (to TR, grant ID:
359 1.1.044.15N, 1.1.044.17N) and an FWO-project (to RP and ME, project ID: G.0A36.15N,
360 G.0521.17N).

361 TR RP ME conceived and designed the study. TR carried out the fieldwork and wrote the first
362 draft. All authors reviewed and revised the manuscript critically and approved the final version.

363

364

References

- 365
366
367 Amlaner, C. J., & Ball, N. J. (1983). A synthesis of sleep in wild birds. *Behaviour*, 87(1), 85-
368 119. doi:10.1163/156853983x00138
- 369 Amo, L., Caro, S. P., & Visser, M. E. (2011). Sleeping birds do not respond to predator
370 odour. *PloS One*, 6(11), e27576. doi:10.1371/journal.pone.0027576
- 371 Arroyo-Solis, A., Castillo, J. M., Figueroa, E., Lopez-Sanchez, J. L., & Slabbekoorn, H.
372 (2013). Experimental evidence for an impact of anthropogenic noise on dawn chorus
373 timing in urban birds. *Journal of Avian Biology*, 44(3), 288-296. doi:10.1111/j.1600-
374 048X.2012.05796.x
- 375 Aulsebrook, A. E., Jones, T. M., Rattenborg, N. C., Roth II, T. C., & Lesku, J. A. (2016).
376 Sleep ecophysiology: Integrating neuroscience and ecology. *Trends in Ecology &*
377 *Evolution*, 31(8), 590-599. doi:10.1016/j.tree.2016.05.004
- 378 Bates, D., Maechler, M., Bolker, B., & Walker, S. (2013). lme4: Linear mixed-effects models
379 using Eigen and S4 (Version R package version 1.0-4). Retrieved from
380 <http://CRAN.R-project.org/package=lme4>
- 381 Casasole, G., Raap, T., Costantini, D., AbdElgawad, H., Asard, H., Pinxten, R., & Eens, M.
382 (2017). Neither artificial light at night, anthropogenic noise nor distance from roads
383 are associated with oxidative status of nestlings in an urban population of songbirds.
384 *Comparative Biochemistry and Physiology. Part A: Molecular and Integrative*
385 *Physiology*, 210, 14-21. doi:10.1016/j.cbpa.2017.05.003
- 386 Christe, P., Richner, H., & Oppliger, A. (1996). Of great tits and fleas: Sleep baby sleep ...
387 *Animal Behaviour*, 52(6), 1087-1092. doi:10.1006/anbe.1996.0256
- 388 Da Silva, A., & Kempnaers, B. (2017). Singing from North to South: Latitudinal variation in
389 timing of dawn singing under natural and artificial light conditions. *Journal of Animal*
390 *Ecology*, 86(6), 1286-1297. doi:10.1111/1365-2656.12739

- 391 Da Silva, A., Samplonius, J. M., Schlicht, E., Valcu, M., & Kempenaers, B. (2014). Artificial
392 night lighting rather than traffic noise affects the daily timing of dawn and dusk
393 singing in common European songbirds. *Behavioral Ecology*, 25(5), 1037-1047.
394 doi:10.1093/beheco/aru103
- 395 Da Silva, A., Valcu, M., & Kempenaers, B. (2015). Light pollution alters the phenology of
396 dawn and dusk singing in common European songbirds. *Philosophical Transactions of
397 the Royal Society of London. Series B: Biological Sciences*, 370(1667), 20140126.
398 doi:10.1098/rstb.2014.0126
- 399 Da Silva, A., Valcu, M., & Kempenaers, B. (2016). Behavioural plasticity in the onset of
400 dawn song under intermittent experimental night lighting. *Animal Behaviour*, 117,
401 155-165. doi:<http://dx.doi.org/10.1016/j.anbehav.2016.05.001>
- 402 de Jong, M., Jeninga, L., Ouyang, J. Q., van Oers, K., Spoelstra, K., & Visser, M. E. (2016).
403 Dose-dependent responses of avian daily rhythms to artificial light at night.
404 *Physiology & Behavior*, 155, 172-179. doi:10.1016/j.physbeh.2015.12.012
- 405 Dominoni, D., Quetting, M., & Partecke, J. (2013). Artificial light at night advances avian
406 reproductive physiology. *Proceedings: Biological Sciences*, 280(1756), 20123017.
407 doi:10.1098/rspb.2012.3017
- 408 Dominoni, D. M., Carmona-Wagner, E. O., Hofmann, M., Kranstauber, B., & Partecke, J.
409 (2014). Individual-based measurements of light intensity provide new insights into the
410 effects of artificial light at night on daily rhythms of urban-dwelling songbirds.
411 *Journal of Animal Ecology*, 83(3), 681-692. doi:10.1111/1365-2656.12150
- 412 Duffy, J. P., Bennie, J., Duran, A. P., & Gaston, K. J. (2015). Mammalian ranges are
413 experiencing erosion of natural darkness. *Scientific Reports*, 5, 12042.
414 doi:10.1038/srep12042

415 Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C. C., Elvidge, C. D., Baugh, K., . . . Furgoni, R.
416 (2016). The new world atlas of artificial night sky brightness. *Sci Adv*, 2(6), e1600377.
417 doi:10.1126/sciadv.1600377

418 Fuller, R. A., Warren, P. H., & Gaston, K. J. (2007). Daytime noise predicts nocturnal singing
419 in urban robins. *Biology Letters*, 3(4), 368-370. doi:10.1098/rsbl.2007.0134

420 Gaston, K. J., Bennie, J., Davies, T. W., & Hopkins, J. (2013). The ecological impacts of
421 nighttime light pollution: a mechanistic appraisal. *Biol Rev*, 88(4), 912-927.
422 doi:10.1111/brv.12036

423 Gaston, K. J., Davies, T. W., Nedelec, S. L., & Holt, L. A. (2017). Impacts of artificial light at
424 night on biological timings. *Annual Review of Ecology, Evolution, and Systematics*,
425 Vol 48, 48(1), 49-68. doi:10.1146/annurev-ecolsys-110316-022745

426 Gil, D., Honarmand, M., Pascual, J., Pérez-Mena, E., & Macías Garcia, C. (2015). Birds
427 living near airports advance their dawn chorus and reduce overlap with aircraft noise.
428 *Behavioral Ecology*, 26(2), 435-443. doi:10.1093/beheco/aru207

429 Gobes, S. M., Zandbergen, M. A., & Bolhuis, J. J. (2010). Memory in the making: localized
430 brain activation related to song learning in young songbirds. *Proceedings: Biological*
431 *Sciences*, 277(1698), 3343-3351. doi:10.1098/rspb.2010.0870

432 Halfwerk, W., & Slabbekoorn, H. (2015). Pollution going multimodal: the complex impact of
433 the human-altered sensory environment on animal perception and performance.
434 *Biology Letters*, 11(4). doi:10.1098/rsbl.2014.1051

435 Hinde, R. A. (1952). The behaviour of the great tit (*Parus major*) and some other related
436 species. *Behaviour. Supplement*, III-201.

437 Hölker, F., Wolter, C., Perkin, E. K., & Tockner, K. (2010). Light pollution as a biodiversity
438 threat. *Trends in Ecology & Evolution*, 25(12), 681-682.
439 doi:10.1016/j.tree.2010.09.007

440 Kempnaers, B., Borgstrom, P., Loes, P., Schlicht, E., & Valcu, M. (2010). Artificial night
441 lighting affects dawn song, extra-pair siring success, and lay date in songbirds.
442 *Current Biology*, 20(19), 1735-1739. doi:10.1016/j.cub.2010.08.028

443 Kluijver, H. N. (1950). Daily routines of the Great Tit, *Parus m. major* L. *Ardea*, 38(3-4), 99-
444 135.

445 Kyba, C. C. M., & Hölker, F. (2013). Do artificially illuminated skies affect biodiversity in
446 nocturnal landscapes? *Landscape Ecology*, 28(9), 1637-1640. doi:10.1007/s10980-
447 013-9936-3

448 Kyba, C. C. M., Kuester, T., Sanchez de Miguel, A., Baugh, K., Jechow, A., Holker, F., . . .
449 Guanter, L. (2017). Artificially lit surface of Earth at night increasing in radiance and
450 extent. *Sci Adv*, 3(11), e1701528. doi:10.1126/sciadv.1701528

451 Navara, K. J., & Nelson, R. J. (2007). The dark side of light at night: physiological,
452 epidemiological, and ecological consequences. *Journal of Pineal Research*, 43(3),
453 215-224. doi:10.1111/j.1600-079X.2007.00473.x

454 R Core Team. (2016). R: A language and environment for statistical computing (Version
455 3.3.2). Vienna, Austria: R Foundation for Statistical Computing. Retrieved from
456 <http://www.R-project.org/>

457 Raap, T., Casasole, G., Costantini, D., AbdElgawad, H., Asard, H., Pinxten, R., & Eens, M.
458 (2016a). Artificial light at night affects body mass but not oxidative status in free-
459 living nestling songbirds: an experimental study. *Scientific Reports*, 6, 35626.
460 doi:10.1038/srep35626

461 Raap, T., Casasole, G., Pinxten, R., & Eens, M. (2016b). Early life exposure to artificial light
462 at night affects the physiological condition: An experimental study on the
463 ecophysiology of free-living nestling songbirds. *Environmental Pollution*, 218, 909-
464 914. doi:10.1016/j.envpol.2016.08.024

465 Raap, T., Pinxten, R., Casasole, G., Dehnhard, N., & Eens, M. (2017a). Ambient
466 anthropogenic noise but not light is associated with the ecophysiology of free-living
467 songbird nestlings. *Scientific Reports*, 7(1), 2754. doi:10.1038/s41598-017-02940-5

468 Raap, T., Pinxten, R., & Eens, M. (2015). Light pollution disrupts sleep in free-living animals.
469 *Scientific Reports*, 5, 13557. doi:10.1038/srep13557

470 Raap, T., Pinxten, R., & Eens, M. (2016c). Artificial light at night disrupts sleep in female
471 great tits (*Parus major*) during the nestling period, and is followed by a sleep rebound.
472 *Environmental Pollution*, 215, 125-134. doi:10.1016/j.envpol.2016.04.100

473 Raap, T., Pinxten, R., & Eens, M. (2017b). Rigorous field experiments are essential to
474 understand the genuine severity of light pollution and to identify possible solutions.
475 *Global Change Biology*, 23(12), 5024-5026. doi:10.1111/gcb.13843

476 Raap, T., Pinxten, R., & Eens, M. (2018). Artificial light at night causes an unexpected
477 increase in oxalate in developing male songbirds. *Conserv Physiol*, 6(1).
478 doi:10.1093/conphys/coy005

479 Raap, T., Sun, J., Pinxten, R., & Eens, M. (2017c). Disruptive effects of light pollution on
480 sleep in free-living birds: Season and/or light intensity-dependent? *Behavioural*
481 *Processes*, 144, 13-19. doi:10.1016/j.beproc.2017.08.011

482 Rattenborg, N. C., de la Iglesia, H. O., Kempnaers, B., Lesku, J. A., Meerlo, P., & Scriba, M.
483 F. (2017). Sleep research goes wild: new methods and approaches to investigate the
484 ecology, evolution and functions of sleep. *Philosophical Transactions of the Royal*
485 *Society of London. Series B: Biological Sciences*, 372(1734), 20160251.
486 doi:10.1098/rstb.2016.0251

487 Rich, C., & Longcore, T. (2005). *Ecological consequences of artificial night lighting* (T. L.
488 Catherine Rich Ed.). Washington: Island Press.

489 Rivera-Gutierrez, H. F., Pinxten, R., & Eens, M. (2010). Multiple signals for multiple
490 messages: great tit, *Parus major*, song signals age and survival. *Animal Behaviour*,
491 80(3), 451-459. doi:10.1016/j.anbehav.2010.06.002

492 Rivera-Gutierrez, H. F., Pinxten, R., & Eens, M. (2012). Tuning and fading voices in
493 songbirds: age-dependent changes in two acoustic traits across the life span. *Animal*
494 *Behaviour*, 83(5), 1279-1283. doi:10.1016/j.anbehav.2012.03.001

495 Roth II, T. C., Rattenborg, N. C., & Pravosudov, V. V. (2010). The ecological relevance of
496 sleep: the trade-off between sleep, memory and energy conservation. *Philosophical*
497 *Transactions of the Royal Society of London. Series B: Biological Sciences*,
498 365(1542), 945-959. doi:10.1098/rstb.2009.0209

499 Ruxton, G., & Colegrave, N. (2010). *Experimental design for the life sciences*. Oxford:
500 Oxford University Press.

501 Schubert, E. F., & Kim, J. K. (2005). Solid-state light sources getting smart. *Science*,
502 308(5726), 1274-1278. doi:10.1126/science.1108712

503 Seltman, H. J. (2013). *Experimental design and analysis* (Vol. 428). Pittsburgh: Carnegie
504 Mellon University.

505 Siegel, J. M. (2009). Sleep viewed as a state of adaptive inactivity. *Nature Reviews:*
506 *Neuroscience*, 10(10), 747-753. doi:10.1038/nrn2697

507 Steinmeyer, C., Mueller, J. C., & Kempenaers, B. (2013). Individual variation in sleep
508 behaviour in blue tits *Cyanistes caeruleus*: assortative mating and associations with
509 fitness-related traits. *Journal of Avian Biology*, 44(2), 159-168. doi:10.1111/j.1600-
510 048X.2012.05750.x

511 Steinmeyer, C., Schielzeth, H., Mueller, J. C., & Kempenaers, B. (2010). Variation in sleep
512 behaviour in free-living blue tits, *Cyanistes caeruleus*: effects of sex, age and
513 environment. *Animal Behaviour*, 80(5), 853-864. doi:10.1016/j.anbehav.2010.08.005

514 Stracey, C. M., Wynn, B., & Robinson, S. K. (2014). Light pollution allows the northern
515 mockingbird (*Mimus polyglottos*) to feed nestlings after dark. *Wilson Journal of*
516 *Ornithology*, 126(2), 366-369. doi:10.1676/13-107.1

517 Stuber, E. F., Baumgartner, C., Dingemanse, N. J., Kempnaers, B., & Mueller, J. C. (2016).
518 Genetic correlates of individual differences in sleep behavior of free-living great tits
519 (*Parus major*). *G3 (Bethesda)*, 6(3), 599-607. doi:10.1534/g3.115.024216

520 Stuber, E. F., Dingemanse, N. J., Kempnaers, B., & Mueller, J. C. (2015a). Sources of
521 intraspecific variation in sleep behaviour of wild great tits. *Animal Behaviour*, 106(0),
522 201-221. doi:10.1016/j.anbehav.2015.05.025

523 Stuber, E. F., Grobis, M. M., Abbey-Lee, R., Kempnaers, B., Mueller, J. C., & Dingemanse,
524 N. J. (2014). Perceived predation risk affects sleep behaviour in free-living great tits,
525 *Parus major*. *Animal Behaviour*, 98(0), 157-165. doi:10.1016/j.anbehav.2014.10.010

526 Stuber, E. F., Mathot, K. J., Kempnaers, B., Dingemanse, N. J., & Mueller, J. C. (2015b).
527 Sex-specific association between sleep and basal metabolic rate in great tits. *Animal*
528 *Behaviour*, 109, 15-22. doi:10.1016/j.anbehav.2015.08.004

529 Sun, J., Raap, T., Pinxten, R., & Eens, M. (2017). Artificial light at night affects sleep
530 behaviour differently in two closely related songbird species. *Environmental*
531 *Pollution*, 231(Pt 1), 882-889. doi:10.1016/j.envpol.2017.08.098

532 Szymczak, J. T., Helb, H. W., & Kaiser, W. (1993). Electrophysiological and behavioral
533 correlates of sleep in the blackbird (*Turdus merula*). *Physiology & Behavior*, 53(6),
534 1201-1210. doi:[https://doi.org/10.1016/0031-9384\(93\)90380-X](https://doi.org/10.1016/0031-9384(93)90380-X)

535 Thys, B., Pinxten, R., Raap, T., De Meester, G., Rivera-Gutierrez, H. F., & Eens, M. (2017).
536 The female perspective of personality in a wild songbird: Repeatable aggressiveness
537 relates to exploration behaviour. *Scientific Reports*, 7(1), 7656. doi:10.1038/s41598-
538 017-08001-1

539 Titulaer, M., Spoelstra, K., Lange, C. Y., & Visser, M. E. (2012). Activity patterns during
540 food provisioning are affected by artificial light in free living great tits (*Parus major*).
541 *PloS One*, 7(5), e37377. doi:10.1371/journal.pone.0037377

542 Tripet, F., Glaser, M., & Richner, H. (2002). Behavioural responses to ectoparasites: time-
543 budget adjustments and what matters to Blue Tits *Parus caeruleus* infested by fleas.
544 *Ibis*, 144(3), 461-469. doi:10.1046/j.1474-919X.2002.00018.x

545 Van Duyse, E., Pinxten, R., & Eens, M. (2000). Does testosterone affect the trade-off between
546 investment in sexual/territorial behaviour and parental care in male great tits?
547 *Behaviour*, 137, 1503-1515.

548 Van Duyse, E., Pinxten, R., Snoeijs, T., & Eens, M. (2005). Simultaneous treatment with an
549 aromatase inhibitor and an anti-androgen decreases the likelihood of dawn song in
550 free-living male great tits, *Parus major*. *Hormones and Behavior*, 48(2), 243-251.
551 doi:10.1016/j.yhbeh.2005.02.013

552 Vermeulen, A., Muller, W., & Eens, M. (2016). Vitrally important - does early innate
553 immunity predict recruitment and adult innate immunity? *Ecology and Evolution*,
554 6(6), 1799-1808. doi:10.1002/ece3.1939

555 Vorster, A. P., & Born, J. (2015). Sleep and memory in mammals, birds and invertebrates.
556 *Neuroscience & Biobehavioral Reviews*, 50(0), 103-119.
557 doi:10.1016/j.neubiorev.2014.09.020

558 Weljie, A. M., Meerlo, P., Goel, N., Sengupta, A., Kayser, M. S., Abel, T., . . . Sehgal, A.
559 (2015). Oxalic acid and diacylglycerol 36:3 are cross-species markers of sleep debt.
560 *Proceedings of the National Academy of Sciences of the United States of America*,
561 112(8), 2569-2574. doi:10.1073/pnas.1417432112

562 Xia, C., Lloyd, H., Shi, J., Wei, C., Zhang, Y., & Manser, M. (2018). Dawn singing of the
563 Brownish-flanked Bush Warbler influences dawn chorusing in a bird community.
564 *Ethology*, 0(0). doi:10.1111/eth.12740

565 Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid
566 common statistical problems. *Methods in Ecology and Evolution*, 1(1), 3-14.
567 doi:10.1111/j.2041-210X.2009.00001.x

568 Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects*
569 *models and extensions in ecology with R*. New York: Springer.

570