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1 **Bats seek refuge in cluttered environment when exposed to white**
2 **and red lights at night**

3

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24

25 **Abstract**

26

27 **Background:** Artificial light at night is recognized as an increasing threat to biodiversity.

28 However, information on the way highly mobile taxa such as bats spatially respond to light is
29 limited. Following the hypothesis of a behavioural adaptation to the perceived risks of
30 predation, we hypothesised that bats should avoid lit areas by shifting their flight route to less
31 exposed conditions.

32 **Methods:** Using 3D acoustic localization at four experimentally illuminated sites, we studied
33 how the distance to streetlights emitting white and red light affected the Probability of bats
34 Flying Inside the Forest (PFIF) *versus* along the forest edge.

35 **Results:** We show that open-, edge-, and narrow-space foraging bats strongly change flight
36 patterns by increasing PFIF when getting closer to white and red streetlights placed in the
37 forest edge. These behavioural changes occurred mainly on the streetlight side where light
38 was directed.

39 **Conclusions:** The results show that bats cope with light exposure by actively seeking refuge
40 in cluttered environment, potentially due to involved predation risks. This is a clear indication
41 that bats make use of landscape structures when reacting to light, and shows the potential of
42 vegetation and streetlight orientation in mitigating effects of light. The study nevertheless
43 calls for preserving darkness as the most efficient way.

44

45 Key words: acoustic localization, artificial light, flight behaviour, chiroptera, microphone
46 array, streetlight

47

48 **1. Background**

49

50 Artificial light at night (ALAN) is recognized as a prominent and growing threat to global
51 biodiversity [1] and hence there is an urgent need to expand scientific knowledge on its
52 effects on ecosystems, and a demand for efficient solutions to reduce these [2]. ALAN
53 impacts a wide range of taxa, at different spatiotemporal scales [3,4]. Effects vary from the
54 individual level to the disruption of ecosystem functioning by altering interactions between
55 species and regulatory processes [5–7].

56 ALAN also affects spatial behaviour by disorienting species and forming barriers in the
57 landscape. For instance, artificial light disorients migrating birds [8] and obstruct toads [9]
58 and highly mobile taxa such as bats [10]. However, the impact of ALAN on species
59 movement across nightscapes remains poorly documented, in particular underlying
60 mechanisms such as changes in spatial behaviour and movement (e.g. flight speed, flight
61 route) of bats in response to light [11] which potentially affects energetic cost and fitness of
62 individuals [12].

63 This topic is all the more important given that bats are mostly nocturnal and well known to be
64 impacted by ALAN in term of activity [13]. Depending on species, ALAN positively or
65 negatively impacts bat activity (e.g. *Pipistrellus* spp and *Nyctalus* spp., respectively *Myotis*
66 spp., *Plecotus* spp. and *Rhinolophus* spp.) at the streetlight scale [14–16], while evidence is
67 also accumulating that ALAN negatively impacts all bat species at larger scales [17–19].

68 ALAN also affects bat movement, for example by keeping individuals from crossing of lit
69 gaps in wooded corridors [10] or lit bridges along waterways [20] in urban environments. It
70 was also shown that different spectra reduce commuting activity along hedgerows and that
71 light shy species switch to the unlit side [21].

72 Among possibilities to reduce these impacts, light spectrum, intensity, directionality, light
73 spill and the duration of lighting are parameters that potentially can be used to reduce negative
74 effects [22]. For instance, light-shy bats such as *Myotis* and *Plecotus* spp. appear to be equally
75 active close to red streetlights and at unlit sites [23]. However, highly light averse species
76 such as *Rhinolophus hipposideros* have shown to avoid all spectra tested for, including red
77 light [21]. Specific part night lighting schemes, with lights turned off from midnight to 5 am,
78 were not found to substantially mitigate effects of light at night as they were still on during the
79 activity peak of bats [14]. Moreover, information on impact distances are also essential to
80 prevent negative effects of lighting setups and allow biodiversity friendly urban planning.
81 However, how the response of bats relates to the distance to light sources – and hence light
82 intensity – is still relatively unknown. Thus far, the only study available on how the response
83 of bats varies with distance reveals clear species dependent differences between 10 and 50 m
84 from a light source [24]. For *Eptesicus serotinus* no difference in effects were shown between
85 0 and 10 m from the streetlight, however strong negative effects were present between 25 and
86 50 m from the streetlight [24]. These findings suggest the response of bats to light is intensity
87 dependent.

88 All these effects of ALAN and possible measures for reduction of impact on bats remain so
89 far mostly studied using activity metrics (i.e. indicators of abundance). However, the level of
90 activity close to a light source does not provide information on how light level affects the
91 behaviour of bats. Indeed, using a single microphone only allows the assessment of average
92 bat activity within an acoustic detection range of approximately five to over 100 m, depending
93 on species specific call amplitude [25].

94 Therefore, to assess respective effects of light intensity and spectrum on bats, there is a need
95 for the assessment of species-specific changes in flight behaviour (e.g. changes in flight

96 paths). Acoustic localization in three dimensions (3D) is an effective tool for the assessment
97 of flight paths [26]. In a study using this technique, authors found that bats reduce flight
98 height and increase flight speed in presence of artificial light [11]. The attraction of insects by
99 light [27] creates foraging opportunities for bats [28] which should cause bats to reduce flight
100 speed [29]. However, studies found that light increases flight speed, probably due to an
101 increased fear of predation [11,20]. An alternative solution to reduce predation risk is to avoid
102 open spaces when exposed to light. Hence, we hypothesize that bats that have the opportunity
103 to fly closer to vegetation (i.e. when flying close to the forest edge) seek shelter in the
104 vegetation while getting closer to lights. This hypothesis is supported by the fact that typically
105 highly light-averse species [14,23,30] such as *Myotis* and *Plecotus* species, that are slow-
106 flying and hence more vulnerable to predation by hawking birds, mostly fly in cluttered
107 habitats (hereafter named narrow-space foragers). We also hypothesize this behavioural
108 response (i.e. flight closer to vegetation) to begin at least 10 metres from the light source as
109 shown by Azam et al.[24]. Indeed, distances from the light source at which behavioural
110 responses (avoidance or attraction) are detected vary according to species, and
111 approximatively lie around 50 m for bat species mostly flying in open space (hereafter named
112 open-space foragers) such as *Eptesicus serotinus*, 10 m for bat species mostly flying at
113 wooded edges (hereafter named edge-space foragers) such as *Pipistrellus* species and at up to
114 25 m for narrow-space foragers such as *Myotis* and *Plecotus* species [24]. Such distance
115 thresholds correspond to light intensities lower than one lux for narrow-space foragers and
116 between one and five lux for open-space and edge-space foragers [24].

117 In this study, we hypothesize the distance dependent behavioural response of open, edge and
118 narrow-space foraging bat species to streetlights emitting different spectra. Specifically, in
119 comparison with unlit sites we expect bats to fly closer to the vegetation when getting closer
120 to the light, and as much for spectral composition close to white. Using 3D acoustic

121 localization near experimental light posts in forest edges, we first investigated the probability
122 of bats flying inside the forest *versus* open habitat in relation with the distance to the light. We
123 studied whether this relationship varies around light posts (e.g. the back- and front side, and
124 above and under the lights) in order to determine the potential of impact reduction by light
125 orientation (i.e. shielding by the light armature).

126

127 **2. Methods**

128

129 *(a) Experimental sites*

130 The study was done at four experimentally illuminated sites in The Netherlands, each with
131 four rows (separated by 204 ± 17 m) with five four-meter-tall lampposts (separated by 25 m
132 and the central one at forest edge) placed perpendicular in forest edge habitat (Fig. 1). Each
133 row was randomly assigned to emit white, green or red light (Fortimo white, ClearField red,
134 and ClearSky green light, Philips, Amsterdam, The Netherlands), with one of the rows kept
135 dark (just poles). In this study, we only used the white, red and dark rows. All lights are
136 switched on at sunset, and off at sunrise since spring 2012. All experimental lights emit light
137 in the full spectrum range at low intensity; green lamps have an increased blue and reduced
138 red light emission, and red lamps have an increased red and reduced blue emission (Fig. 1).
139 All light colours have negligible UV emission (see Spoelstra et al. [31] for spectral
140 compositions). The light beam of each light is directed downwards by Philips Residium
141 FGS224 (1xPL-L36WHFP) armatures to project light in preferential directions. The light
142 intensity at ground level is on average 8.7 ± 3.0 lux, which is comparable to the illumination
143 levels of countryside roads (Fig. 1; see Spoelstra et al. [31] for a further description of these
144 experimental sites). For more detail about light intensity in relation with the distance to the
145 lamp and the orientation of the lamp, see Additional file 1, Appendix S1.

146

147 *(b) Sampling design and 3D acoustic localization*

148 Bats were acoustically tracked in three dimensions during the first three hours after sunset for
149 12 nights between the 10th and 22nd of July 2018. As we could deploy only one microphone
150 array at the time, we unable to simultaneously sample different light treatments, so we
151 sampled white light, red light and dark control during separate nights. In order to limit
152 variation in bat behaviour linked with inter-night environmental variations, we always
153 sampled a different light colour between consecutive nights (Additional file 1: Table S1). All
154 nights were sampled under highly favourable and constant temperatures (average=16.2 °C,
155 Standard Deviation=1.7°C) and wind speed conditions (average=1.3 m/s, SD=0.8 m/s)
156 (Additional file 1: Table S1). In total four dark control, four red and four white lights were
157 sampled. White light, red light, and dark control were uniquely sampled in two of the four
158 experimental sites, and combinations of the two spectra (dark control and red, and dark
159 control and white, respectively) were sampled in the two other experimental sites. Within the
160 row of light posts, we always sampled the light post right in the forest edge (i.e. at the border
161 of the forest and the open area; see Fig. 1).

162 To reconstruct 3D positions of bats, we used a trajectography system (hereafter named
163 microphone array) designed at the Institut Langevin (Paris, France). The system uses
164 echolocation calls recorded in a frequency range from eight to 160 kHz at four microphones
165 (FG 3329, Knowles Acoustics, Itasca, IL USA; see Additional file 1, Appendix S2 for more
166 details about sound recording and triggering of echolocation calls). Microphones were
167 arranged in a horizontal triangle form (i.e. three in the corners and one in the middle; Fig. 1).
168 The microphone array was set up similarly near each row: the four microphones were placed
169 in a horizontal plane above the ground surface, with the central microphone in the open space
170 at four meters horizontal distance to the forest edge and to the streetlight axis perpendicular to

171 the forest edge. The microphone array was always placed on the same side of the light (Fig.
172 1). Bat positions in the detection range of the microphones were continuously assessed using
173 the time of arrival difference (TOAD) of bat echolocation calls between microphones in the
174 array, using the call waveform [26]. Further details about the recording setup, the conversion
175 of bat call arrival times into 3D positions, assigning positions to trajectories and the precision
176 of these can be found in Ing et al. [32] and Additional file 1, Appendices S2 and S3.

177

178 *(c) Calculation of the distance from 3D positions to the light and accounting for*
179 *imprecision*

180 To calculate the distance between each bat position and the light, we used the following
181 equation (D , equation 1):

$$182 \quad D_i = \sqrt{(x_i - 4)^2 + (y_i - 4)^2 + (z_i - 4)^2} \quad (\text{Eq. 1})$$

183 where x , y and z represent distances to the microphone array for each of the three-dimension
184 axis of a given position i . The microphone height was corrected for by entering the actual
185 height of the microphones as placed in the field (i.e. 0.82 to 1 meter) in the position
186 calculation software. Since lights were located at the forest edges and were at four meters
187 height, and given the microphone array placement, we subtract four meters to x , y and z axis,
188 in order to compute the real distance to the light (Fig. 1).

189 Since the imprecision was expected to increase with the distance to the microphone array, we
190 calculated the cumulated imprecision of 3D positions (I , equation 2) as follows:

$$191 \quad I_i = \sqrt{(dx_i)^2 + (dy_i)^2 + (dz_i)^2} \quad (\text{Eq. 2})$$

192 where dx , yx and zx represent the standard deviation of distances to the microphone array
193 estimated for each of the three-dimension axis of a given position i [32]. We choose to discard
194 positions with a cumulated imprecision of more than one meter (Fig. S1) and those not
195 included in any bat individual trajectory (i.e. composed of several positions; for details on

196 trajectory reconstruction see Additional file 1: Appendix S3), which led us to keep 28,646
197 positions on the 35,067 recorded.

198

199 *(d) Assigning species to 3D positions*

200 Individual bat calls used to reconstruct 3D positions were saved by continuously recording
201 sound files. In a second step, sound files were segmented into five-second intervals which is
202 sufficient to cover the average duration of a bat pass [33]. Each of 25,195 five-second files
203 were then classified to the closest taxonomic level using the Tadarida software [34]. Because
204 the identification by echolocation to the species level is difficult, we limited identification to
205 following species groups: the *Eptesicus/Nyctalus* group including *Eptesicus* sp. and *Nyctalus*
206 sp., the *Myotis/Plecotus* group including *Myotis* sp. and *Plecotus* sp., and the *Pipistrellus*
207 group including *Pipistrellus* sp. These three groups respond differently to light:
208 *Eptesicus/Nyctalus* group (i.e. open space forager) are usually considered as light
209 opportunistic or light shy (context dependent), species in the *Myotis/Plecotus* group (i.e.
210 narrow space forager) are light shy, and species in the *Pipistrellus* group (i.e. edge space
211 forager) are light opportunistic.

212 In a third step, we linked the 3D positions in each 5 second file to the species group found by
213 Tadarida. In case calls of different species groups were found within the same 5 second file,
214 we were able to assign the correct species group to separate series of calls by making use of
215 sequential 3D positions and the peak frequency.

216

217 *(e) Statistical analysis*

218 We assessed whether the probability of bats flying inside the forest (PFIF) differed according
219 to the distance to the light, and whether this relationship differed between spectra (i.e. dark
220 control, red and white lights). The relationship between the PFIF and the distance to the light

221 allowed us to define the Flight-Path Switch Distance (FPSD) as the distance at which bats on
222 average flew as much inside as outside the forest. We performed Generalized Linear Mixed
223 Models (GLMM, R package *TMB*), using the PFIF as a binomial response variable where
224 zero corresponded to positions located in the open habitat, and where one corresponded to
225 positions located inside the forest (Fig. 1). We used as explanatory variables the distance to
226 the light, the spectrum type, and the interaction between them to assess the effect of spectra
227 on FPSD. To account for a part of the pseudo-replication (i.e. an average of 15.4 ± 10.1
228 positions per trajectory; Additional file 1: Fig. S2), we included a random effect on the
229 trajectory identifier. We also included the date as random term in models to control for
230 potential inter-site (i.e. one site sampled each night) and inter-night variations of bat
231 behaviour in relation with lights. Note that weather conditions were highly favourable to bats
232 and stable throughout the sampling period, and that habitat composition was similar between
233 sites (see Sampling design and 3D acoustic localization section and Additional file 1: Table
234 S1).

235 Given that imprecisions of positions were slightly positively correlated with the distance to
236 the light for *Eptesicus/Nyctalus* group ($r = 0.05$, $t = 2.0$, $df = 1786$, $p\text{-value} < 0.045$; Pearson's
237 correlation test), we adapted the weight of response variables to the associated precision of
238 positions (i.e. inverse of the imprecision squared [35]) by adding a precision weight term in
239 GLMMs.

240 Lights were oriented toward the ground and the armature parallel to the forest edge, which
241 results in a heterogeneous distribution of light in horizontal and vertical planes (Fig. 1). Thus,
242 in order to assess the dependence of light effects on bats to their spatial position around a
243 streetlight, we built one model per species group for (i) all positions around the light, for (ii)
244 positions under the light (i.e. $z < 4$ m), for (iii) positions above the light ($z > 4$ m), for (iv)
245 positions at the backside of the light (i.e. $x > 4$ m) and for (v) positions in front of the light

246 (i.e. $x < 4$ m) (Fig.1). All GLMMs exhibited much smaller Akaike Information Criteria (AIC)
247 than null models. We assessed for each model the goodness of fit by computing the
248 percentage of variance explained by models using the r^2 function (R package *sjstats*). We
249 checked residual plots of models using the R package *DHARMA*. All analyses were performed
250 using a significance threshold of 5% in the R statistical software [36].

251

252 **3. Results**

253

254 ***(a) 3D acoustic localization***

255 Bat calls within the 25,195 five-second files recorded allowed the assessment of a total of
256 28,646 (3D) positions, with an imprecision of less than one meter. Of all positions, 91.3 %
257 were assigned to the *Pipistrellus* group, 6.2 % to the *Eptesicus/Nyctalus* group and 2.4 % to
258 the *Myotis/Plecotus* group (Additional file 1: Table S2). The number of locations was higher
259 at the white-light poles, followed by red and then dark control poles for *Eptesicus/Nyctalus*
260 and *Pipistrellus* groups, but higher around red-light poles followed by white and then dark
261 control poles for *Myotis/Plecotus* group (Additional file 1: Table S2). Overall, the cumulative
262 imprecision for each location varied between 0.10 and 0.39 m on average, and was dependent
263 on species group, but similar between the three light treatments although slightly lower for all
264 groups in red sites (Additional file 1: Table S2). More than 70 % of positions had a
265 cumulative imprecision lower than 0.2 meters (Fig. S1).

266

267 ***(b) Effect of spectrum on the flight behaviour***

268 Overall, the average probability of bats flying inside the forest (PFIF) was significantly higher
269 near red and white light posts compared to dark control poles for *Pipistrellus* group, while
270 only significantly higher near white light posts for *Myotis/Plecotus* and *Eptesicus/Nyctalus*

271 groups (Table 1). Then, all bat groups were found to have a greater PFIF when getting closer
272 to the light. The increase in PFIF when getting closer to the light was stronger for red and
273 white light posts compared to dark control poles for *Pipistrellus* and *Eptesicus/Nyctalus*
274 groups, and only stronger for white light posts for the *Myotis/Plecotus* group. The increase in
275 PFIF when getting closer to the light was even stronger for red compared to white light posts
276 for the *Pipistrellus* group, and even stronger for white compared to red light posts for
277 *Eptesicus/Nyctalus* (Table 1; Fig. 2). Irrespective of these differences, white lighting
278 increased the Flight Path Switch Distance (FPSD) for *Eptesicus/Nyctalus* group (i.e. 6.1 m),
279 red lighting generated a greater FPSD for *Pipistrellus* group (i.e. 2.0 m), and white light
280 generated a FPSD of 5.5 m for *Myotis/Plecotus* group (Additional file 1: Table S3; Fig. 2).

281 At three to five metres from the light, the PFIF for *Myotis/Plecotus* and *Eptesicus/Nyctalus*
282 groups even reached 100 % for white lighting treatment, while the usual PFIF at such distance
283 in unlit sites was under 1 % (Fig. 2). Similarly, the PFIF for *Pipistrellus* group reached more
284 than 85 % and 50 % at one meter from red and white lights respectively, while close to 0 % in
285 unlit conditions whatever the distance (Fig. 2).

286

287 ***(c) Variation of responses according to location around lights***

288 All species groups increasingly flew inside the forest when getting closer to the light. For both
289 spectra, this effect was only present at the front side of the light, except for *Eptesicus/Nyctalus*
290 group around red lights (Table 1; Fig. 3). For this group, the response was furthermore limited
291 for bats flying above light posts of both spectra. For the *Pipistrellus* group, this response was
292 unrelated to flight height (Table 1; Fig. 3).

293 Concerning the distance dependency, Flight Path Switch Distance (FPSD) was greater for
294 *Eptesicus/Nyctalus* flying above and at the front side of white compared to red light poles (5.1
295 m and 7.0 m *versus* 4.0 m and 0.2 m, respectively), and was greater for *Myotis/Plecotus* flying

296 at the front side of white light poles (6.7 m *versus* no prediction possibility due to insufficient
297 PFIF, respectively) (Additional file 1: Table S3; Fig. 3). The FPSD was also higher under and
298 at the front side of red-light poles compared to white light poles for *Pipistrellus* group (3.2 m
299 and 2.4 m *versus* no prediction possibility due to insufficient PFIF, respectively) (Additional
300 file 1: Table S3; Fig. 3).

301 Finally, fixed effects of models overall almost always explained a large part of the variance
302 (0.11-0.90 r squared; Additional file 1: Table S3).

303

304 **4. Discussion**

305

306 We show that artificial light located at forest edges significantly increases the Probability of
307 Flying Inside the Forest (PFIF) for open-, edge- and narrow-space foragers
308 (*Eptesicus/Nyctalus*, *Pipistrellus* and *Myotis/Plecotus* groups, respectively) regardless of light
309 spectrum.

310 For open- and edge-space foraging bats that take advantage of around light accumulated
311 insects, the presence of cluttered habitat (i.e. forest in our case) could further facilitate
312 foraging around streetlights by providing shelter against predators. This result is also
313 consistent with the antagonist effects of ALAN at different spatial scales for open- and edge-
314 space foragers. At the streetlight scale these groups can appear light-opportunistic
315 [13,21,23,24,37], however, at a larger scale these species are negatively impacted by ALAN
316 [17–19]. The observation that light-opportunistic open- and edge-space foragers seek refuge
317 in cluttered environment near light sources may explain this negative impact, especially in
318 areas with little vegetation around light.

319 Comparable behavioural changes were expected for *Myotis/Plectus* species as they are
320 adapted to fly in cluttered environments and are known to be light-averse [14,23,30]. Both red
321 and white light increased the PFIF for *Myotis/Plecotus* group compared to dark control sites.
322 Although the effect of red lights was much less important compared to white lights, this
323 finding is important as red light has been reported to have limited to absent effects on the
324 activity of these species [21,23]. Our results thus suggest that red light may actually not be
325 entirely effective in avoiding behavioural changes of narrow-space foragers, and even less for
326 open- and edge-space foragers.

327 Overall, Flight Path Switch Distances (FPSD) were mostly longer around white lights, which
328 is likely due to the fact that bats may perceive white light as more intense compared to red
329 light due their spectral sensitivity [38,39]. Such differences in FPSD could also be linked with
330 differences in light intensity at equal distance, higher for white than red lamps we studied
331 (4.83 more lux in average in a 5 m radius around red lights; see Additional file 1: Appendix
332 S1 for graphical representation of light intensity in relation to the distance to white and red
333 lights), which is known to be one of light parameters driving impacts on bats [40]. When
334 considering the vertical location of bat positions, we found that compared to white light poles,
335 the FPSD was higher for individuals located under (i.e. for *Pipistrellus* group) and above (i.e.
336 for *Pipistrellus* and *Eptesicus/Nyctalus* groups) red light poles. This is likely directly related
337 to the distribution of the light around the streetlights (supplementary material S1), and aligns
338 with the distance relation – and hence intensity dependence – of activity reported by [24].
339 However, it should be noted that *Eptesicus/Nyctalus* group mainly flew above lights
340 (Additional file 1: Table S2) which likely explain the absence of response under lights.

341 We also found changes in bat behaviour in front of light posts for all groups but not at the
342 backside. Thus, the directionality of the light post matters and can be used to reduce the
343 adverse impacts of artificial lighting on bats. In our study, we had a sharper cut-off in light at

344 the backside of the light posts, and hence the effects there disappeared at shorter distances.
345 Individuals could forage at the backside of streetlights where the predation risk is low, and
346 hence not seek refuge inside forest when getting closer to the light. Further investigations are
347 needed to understand mechanisms involved. Concerning the overall higher effect of red light
348 compared to white light on *Pipistrellus* group, further studies would be needed to understand
349 why, and if they possibly turn back when getting closer to light instead of seeking refuge
350 inside the forest. However, open-space foragers do not show the same pattern and react
351 similarly to red and white light. We could hypothesise that their higher flight height allows for
352 flying in or above the canopy (i.e. in a potentially more open space than for *Pipistrellus*
353 group) while increasing their flight speed in response to light, which could explain their
354 different response than edge-space foragers. Further investigations are also needed to address
355 these aspects.

356 Finally, depending on bat location around streetlights, Flight Path Switch Distances (FPSD) in
357 front of streetlights overall started from 7 m and 4 m for white and red lights, respectively.
358 These distances of impact correspond to light intensities around 6 lux for both white and red
359 lights. However, we defined FPSD as the distance at which bats on average flew as much
360 inside as outside the forest, but impacts likely start before this arbitrarily chosen threshold.
361 When we look at the beginning of behavioural perturbation, i.e. when the PFIF previously
362 close to zero increases towards positive PFIFs, corresponding FPSD would be around 15 m
363 and 9 m for white and red lights, respectively. Such distances correspond to light intensities
364 around one lux for both white and red lights. These thresholds seem to be consistent with a
365 study which looked at thresholds in light intensity affecting bat activity [24].

366 However, it is important to be cautious about the definition of safety thresholds for bats and
367 further studies should confirm these results by testing wider distance and intensity ranges
368 around streetlights, by sampling all spectra simultaneously, and by studying more sites and

369 nights per spectrum. We were not able to measure forest height, thus further studies could
370 accurately account for bat position in relation to forest canopy as open space foragers such as
371 *Nyctalus* spp. can fly above (average flight height 9 ± 4 m; Additional file 1: Fig.S3). However,
372 considerably lower flight heights recorded for *Pipistrellus* (5.8 ± 2.8 m) and *Myotis/Plecotus*
373 groups (5.2 ± 2.5 m), and light effects generalized to under and above light positions for
374 *Pipistrellus* group support these findings (Additional file 1: Fig.S3).

375

376 **5. Conclusion**

377

378 Our study demonstrates that spectrum type, intensity and directionality of streetlights has an
379 effect on the flight behaviour of all bats, including light-opportunistic species, highlighting
380 the need to consider simultaneously all these characteristics when studying ALAN impact on
381 bats. In contrast to the absence of changes in bat activity in response to red light reported
382 earlier, we here show that bats can have a comparable change in flight behaviour in response
383 to red and white light. This finding first shows that bats actively seek refuge in cluttered
384 environment when getting closer to light sources. This is a clear indication that bats make use
385 of landscape structures when dealing with light, and shows the potential of vegetation in
386 mitigating negative impacts of artificial light at night, but calls for preserving darkness as the
387 most efficient way.

388

389 **Ethics approval and consent to participate**

390 Not applicable.

391

392 **Consent for publication**

393 Not applicable.

394

395 **Availability of data and materials**

396 R scripts and data used for analyses are available in the Zenodo repository,

397 <https://doi.org/10.5281/zenodo.4036279>.

398

399 **Competing interests**

400 We declare we have no competing interest.

401

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409

410 **Author's contribution**

411 K.B., I.L., C.K, and K.S. conceived the ideas and designed the methodology; R.K.I. built the

412 microphone array; K.B. and K.S. collected the data, K.S. set up experimentally illuminated

413 sites; K.B. and Y.B. analysed the data and wrote R scripts; K.B, I.L, C.K. and K.S interpreted

414 results; all authors led the writing of the manuscript. All authors contributed to the drafts and

415 gave their final approval for publication.

416

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422 work.

423

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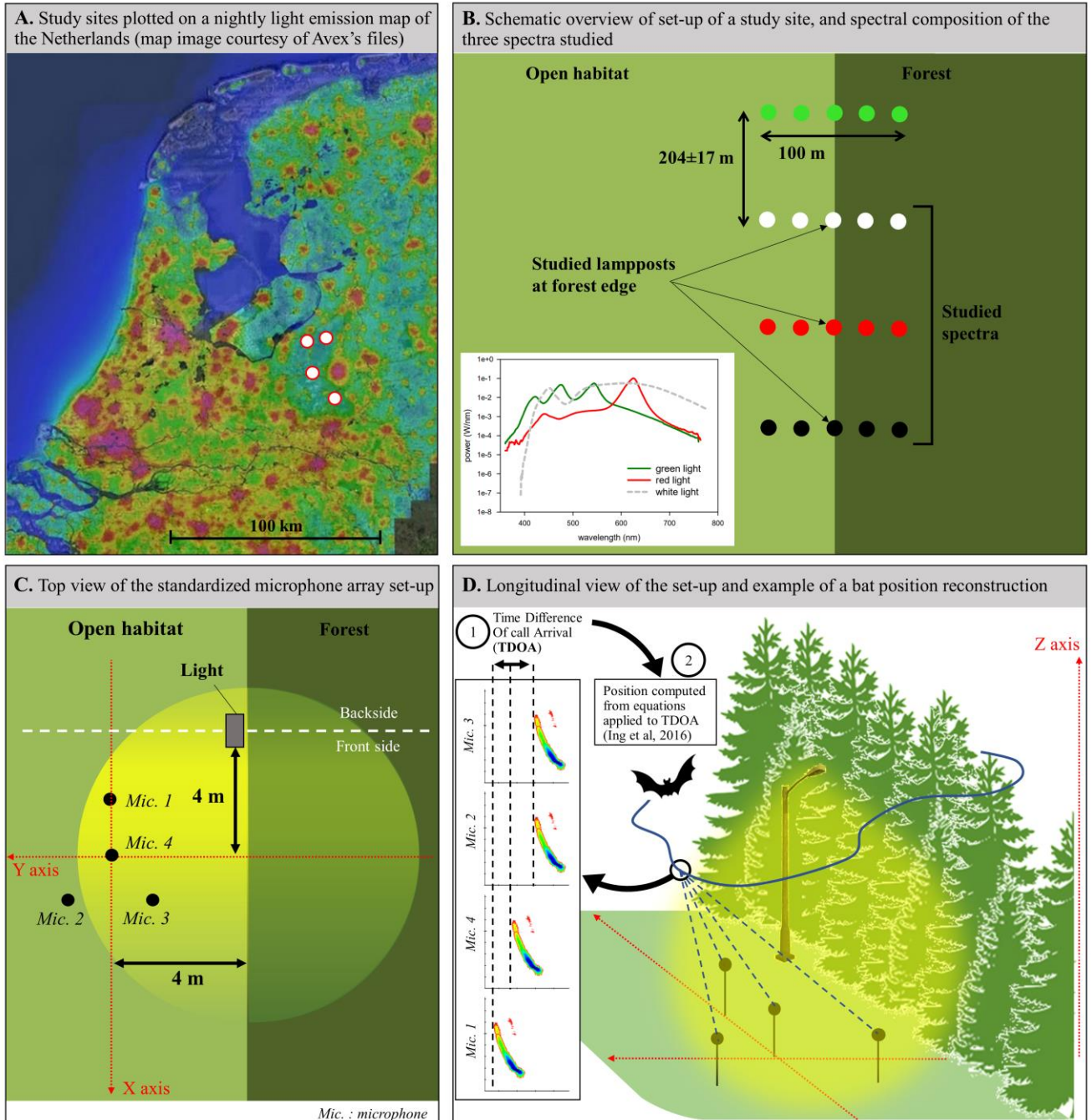
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- 540
- 541

542 **Table 1.** Estimates, standard errors and p-values of the effect of the distance to the light, the
543 spectrum and the mutual interaction on the probability of bats flying inside the forest when
544 unlit control (A) and white spectrum (B) were used as intercept (**p < .001, *p < .01, *p <
545 .05). Results are presented for all positions, and positions above, under, behind and in front of
546 the light (see Fig. 1 for placement definitions), and derived from generalized linear mixed
547 models.

		<i>Eptesicus/Nyctalus</i>	<i>Myotis/Plecotus</i>	<i>Pipistrellus</i>
All positions		N=1,788	N=692	N=26,166
Dist. to light		-0.950 ± 0.020 ***	-1.864 ± 0.736 *	-0.188 ± 0.036 ***
Spectrum	(A) Unlit vs.Red	-0.679 ± 4.278	3.056 ± 4.055	5.185 ± 1.570 ***
	(A) Unlit vs.White	18.890 ± 4.004 ***	16.736 ± 7.994 *	3.522 ± 1.491 *
	(B) White vs.Red	-19.569 ± 3.502 ***	-13.680 ± 8.379	1.663 ± 1.509
Dist. to light : Spectrum	(A) Unlit vs.Red	-1.013 ± 0.044 ***	-0.508 ± 0.376	-0.802 ± 0.067 ***
	(A) Unlit vs.White	-1.639 ± 0.435 ***	-1.537 ± 0.742 *	-0.385 ± 0.040 ***
	(B) White vs.Red	0.625 ± 0.055 ***	1.029 ± 0.790	-0.417 ± 0.059 ***
Vertical location: above light		N=1,708	N=465	N=19,438
Dist. to light		-0.951 ± 0.020 ***	-1.715 ± 794 *	-0.207 ± 0.038 ***
Spectrum	(A) Unlit vs.Red	37.339 ± 8.043 ***	-4.277 ± 6.453	2.587 ± 1.690
	(A) Unlit vs.White	20.557 ± 3.787 ***	7.801 ± 8.163	2.721 ± 1.596 .
	(B) White vs.Red	16.784 ± 8.092 *	-12.078 ± 8.351	-0.134 ± 1.623
Dist. to light : Spectrum	(A) Unlit vs.Red	-6.282 ± 0.187 ***	0.783 ± 0.914	-0.477 ± 0.074 ***
	(A) Unlit vs.White	-1.487 ± 0.044 ***	-0.507 ± 1.059	-0.369 ± 0.043 ***
	(B) White vs.Red	-4.795 ± 0.190 ***	1.290 ± 0.856	-0.108 ± 0.066
Vertical location: under light		N=80	N=227	N=6,728
Dist. to light		/	/	-0.567 ± 0.043 ***
Spectrum	(A) Unlit vs.Red	/	/	10.751 ± 2.080 ***
	(A) Unlit vs.White	/	/	5.340 ± 1.944 **
	(B) White vs.Red	/	/	5.411 ± 1.480 ***
Dist. to light : Spectrum	(A) Unlit vs.Red	/	/	-1.624 ± 0.197 ***
	(A) Unlit vs.White	/	/	-0.581 ± 0.162 ***
	(B) White vs.Red	/	/	-1.044 ± 0.128 ***
Horizontal location: backside		N=895	N=326	N=6,433
Dist. to light		-0.520 ± 0.380	/	-0.347 ± 0.548
Spectrum	(A) Unlit vs.Red	-47.725 ± 29.775	/	-10.136 ± 7.872
	(A) Unlit vs.White	-13.521 ± 7.152 .	/	-5.289 ± 7.145
	(B) White vs.Red	-34.205 ± 28.608	/	-4.847 ± 3.978
Dist. to light : Spectrum	(A) Unlit vs.Red	1.977 ± 1.134 .	/	0.715 ± 0.588
	(A) Unlit vs.White	0.676 ± 0.408 .	/	0.429 ± 0.559
	(B) White vs.Red	1.302 ± 1.026	/	0.286 ± 0.240
Horizontal location: front side		N=893	N=366	N=19,733
Dist. to light		-0.748 ± 157 ***	0.959 ± 0.602	-0.555 ± 0.021 ***
Spectrum	(A) Unlit vs.Red	1.082 ± 3.269	18.784 ± 9.528 *	7.343 ± 1.893***
	(A) Unlit vs.White	6.229 ± 2.215 **	45.356 ± 20.498 *	4.534 ± 1.818 *
	(B) White vs.Red	-5.147 ± 3.384	-26.572 ± 19.127	2.809 ± 1.779
Dist. to light : Spectrum	(A) Unlit vs.Red	-0.538 ± 0.326 .	-2.587 ± 1.036 *	-1.070 ± 0.079 ***
	(A) Unlit vs.White	-0.558 ± 0.170 **	-5.466 ± 2.590 *	-0.468 ± 0.047 ***
	(B) White vs.Red	0.020 ± 0.338	2.879 ± 2.555	-0.602 ± 0.070 ***

549 **Figure 1.** Location of study sites plotted on a nightly light emission map (A), schematic
 550 overview of set-up of a study site (B) and standardized set-up of the microphone array (C),
 551 and how 3D positions are calculated (D). Light posts were always 4 m tall, and always
 552 oriented toward the microphone array, parallel to the forest edge.

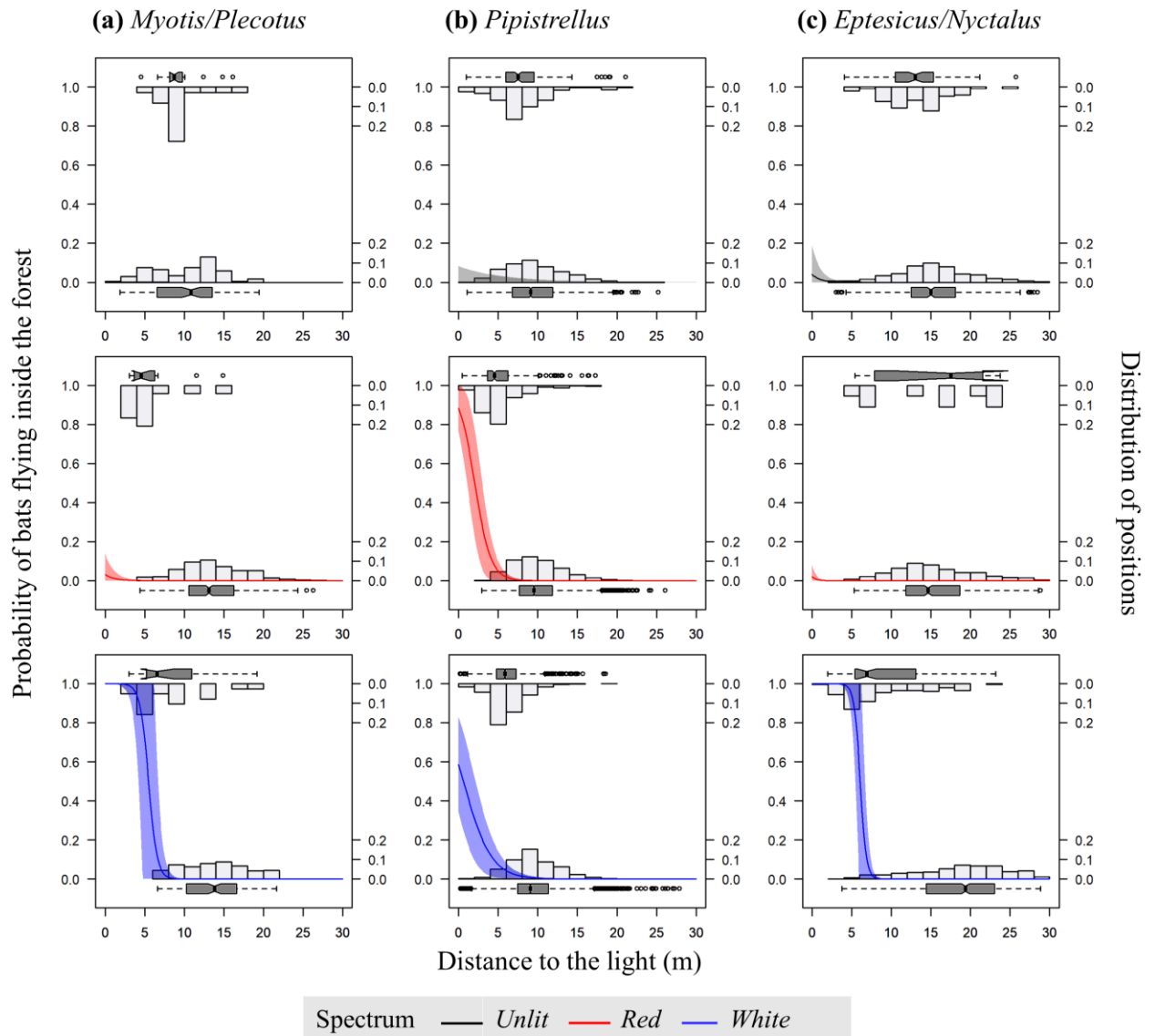


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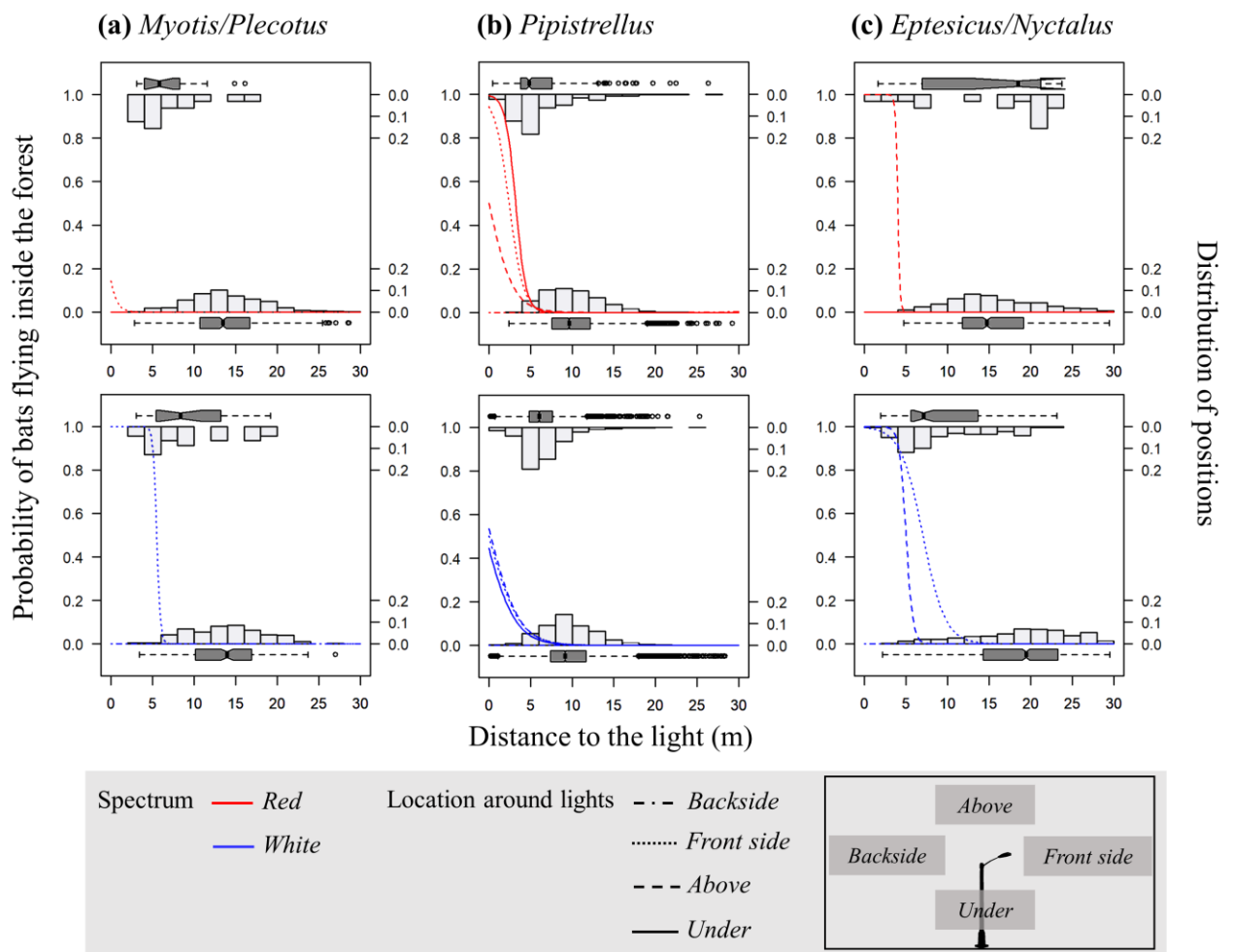
556 **Figure 2.** Predicted curves of the probability of bats flying inside the forest (left y axis) in
 557 relation with the distance to the light for unlit control sites (with dummy light posts), red lit
 558 sites and white lit sites. Histograms and boxplots represent the frequency distribution of bat
 559 positions (right y axis) for positions located in open area (at the bottom) and inside the forest
 560 (at the top) in relation with the distance to the light.



561

562

563 **Figure 3.** Predicted curves of the probability of bats flying inside the forest (left y axis) in
 564 relation with the distance to the light for positions at the backside of streetlight, at the front
 565 side of streetlight, above and under streetlight for red and white lit sites. Histograms and
 566 boxplots represent the frequency distribution of bat positions (right y axis) for positions
 567 located in open area (at the bottom) and inside the forest (at the top) in relation with the
 568 distance to the light.



569