

1 **Influence of local landscape and time of year on bat-road collision risks**

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16 **Abstract**

17 Roads impact bat populations through habitat loss and collisions. High quality habitats particularly  
18 increase bat mortalities on roads, yet many questions remain concerning how local landscape features  
19 may influence bat behaviour and lead to high collision risks (e.g. influence of distance to trees, or of  
20 vegetation density). Moreover, when comparing the potential danger of different road sections, the most  
21 popular method today is the use of simple bat detectors to assess the local densities of current populations

22 at road sites. Yet, it is not known to which extent bat behaviour influences collisions (i.e. bats flying at  
23 vehicle height or on the side or above, bats avoiding vehicles or not). Behaviour is very rarely taken into  
24 account in practice, and this might lead to hazardous site selections for mitigation. Our goals were thus  
25 (i) to estimate how local landscape characteristics affect each of the conditional events leading to  
26 collisions (i.e. bat presence, flight in the zone at collision risk and bat-vehicle co-occurrence), and (ii)  
27 to determine which of the conditional events most contributed to collisions risks.

28 In this study, we recorded bat activity and characterised flight behaviour with three variables: position  
29 at collision risk, bat-vehicle co-occurrence, and flight path orientation, using acoustic flight path tracking  
30 at 66 study sites in the Mediterranean region for two to five full nights. We modelled the effect of the  
31 local landscape, i.e. in a radius of 30 m around the road (vegetation height, distance, density and  
32 orientation), road features (road width, traffic volume) and the time of year on eleven species or species  
33 groups. We built models for each conditional probability of the road collision risk (i.e. species density,  
34 presence in the zone at risk, bat-vehicle co-occurrence) and multiplied their estimates to calculate the  
35 overall collision risk.

36 Our results show that the local landscape had different effects on bat density and presence in the zone  
37 at collision risk. Increasing distance to trees and decreasing tree height were associated with a decrease  
38 in bat density at roads. Forests were the local landscapes where bats flew more often in the zone at  
39 collision risk. The overall collision risk was higher either in forests or at tree rows perpendicular to the  
40 road depending on species. Contrary to common preconceptions, mid-range echolocators seemed to be  
41 generally more at risk of collision than short-range or long-range echolocators. In addition, collision risk  
42 was maximal in summer or autumn for most species. Finally, bats mainly followed the road axis even  
43 when trees were present on both sides or absent.

44 Our results contribute to a better understanding of bat movements in different local environments at the  
45 scale where they directly sense their surroundings with echolocation calls. Disentangling bat density  
46 from flight behaviour allowed us to better understand the temporal and spatial contributors of roadkills,  
47 and to provide guidance for road impact assessment studies.

## 49 **Introduction**

50 Highways and main or secondary roads cover large surfaces of industrialised countries worldwide while  
51 road construction and traffic density rise continuously (Ibisch et al., 2016; van der Ree et al., 2015a).  
52 Both networks lead to troubling impacts on wildlife, namely death by collision, loss of habitat amount  
53 and quality, population fragmentation, which in turn lead to negative impacts on population survival in  
54 numerous taxa (Rytwinski and Fahrig, 2015).

55 To explain the direct ecological impact of roads, i.e. mortality by collision, several studies have  
56 investigated the role of road and land features on roadkill occurrence. They showed for example that  
57 increasing road width, traffic and/or speed limit enhances collisions in large mammals (Nelli et al., 2018;  
58 Neumann et al., 2012; Seiler, 2005; Valero et al., 2015) but the results concerning the role of traffic or  
59 speed limit on road-kills were contrasted for other vertebrate taxa (Clevenger et al., 2003; D'Amico et  
60 al., 2015; Mazerolle, 2004). Studies on a variety of animal groups also found that habitats favourable  
61 for species foraging or movement, described at the home-range scale (e.g. presence or absence of  
62 woodland, cropland, wetland ...), are more often associated with the occurrence of road-kills (Grilo et  
63 al., 2016; Gunson et al., 2011; Malo et al., 2004).

64 Very few studies have investigated the effect of local landscape features (i.e. within the few meters on  
65 either side of the road) on collisions. However, when mitigation measures are recommended, they often  
66 deal with the vegetation structure at this local scale (van der Ree et al., 2015b). Indeed, it is likely that  
67 the landscape in the immediate vicinity of roads affects animal movement trajectories – and, as a result,  
68 the risk of collisions. In ungulates, Meisingset et al. (2014) found that increasing road edge clearance  
69 decreased the rate of collisions. However, as the authors suggest, this is probably partly a driver effect  
70 since drivers benefitting from a better visibility will in all likelihood have more time to avoid collisions.  
71 Large animals that may be avoided by drivers represent only a very small percentage of the species  
72 impacted by collisions (D'Amico et al., 2015; Rytwinski and Fahrig, 2015) and the effects of local  
73 landscapes are likely to be species dependent, but knowledge is very scarce at the species level.

74 The movement of aerial animals is expected to be particularly conditioned by height, density and spatial  
75 arrangement of three-dimensional structures (Brigham et al., 1997; Norberg, 1994, 1986). For example  
76 in birds, gaps in vegetation are an important factor of road collisions (Lin, 2016; Orłowski, 2008).  
77 Among aerial animals impacted by road collisions, bats are long-lived mammals with a low reproductive  
78 rate, having one offspring – exceptionally two – per year (Dietz et al., 2009). Additionally, temperate  
79 bats have suffered from an important decline of their populations in the second half of the twentieth  
80 century, which translates into a poor conservation status today (Van der Meij et al., 2015), and North-  
81 American bats have experienced dramatic declines due to white-nose syndrome, a fungal disease  
82 (Langwig et al., 2015). For these reasons, even moderate increases in mortality rates may represent a  
83 serious threat to their survival. As a result, all European bats are now under strict protection (*Council*  
84 *Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora*, 1992).

85 Bat mortality on roads was investigated in numerous studies (Fensome and Mathews, 2016), and can  
86 locally threaten bat populations. For instance, an annual highway mortality of 5% was estimated for a  
87 colony of *Myotis sodalis* in the United States of America (Russell et al., 2009). Brinkmann et al. (2012)  
88 state that a road mortality of 3 to 7 adult females in a colony of 100 female *M. myotis* or *Rhinolophus*  
89 *hipposideros* could lead to a negative population growth. A good understanding of the mechanisms  
90 leading to collisions between road vehicles and bats is therefore necessary to efficiently mitigate them  
91 (Fensome and Mathews, 2016).

92 At the home range scale, several studies showed that collisions involving bats are concentrated in  
93 habitats qualified as favourable for foraging and commuting (e.g. water bodies, forests and riparian  
94 habitats) (Gaisler et al., 2009; Lesiński, 2007; Medinas et al., 2019, 2013). At the local scale, it is  
95 suspected that the orientation of tree lines and vegetation structure (i.e. height, density and distance from  
96 road edge) direct bat movement, and are consequently major factors of road collision risks (Fensome  
97 and Mathews, 2016). The influence of vegetation structure on bat activity has been relatively well  
98 studied in the literature (Kelm et al., 2014; Pourshoushtari et al., 2018; Toffoli, 2016; Verboom and  
99 Spoelstra, 1999). However, the influence of local landscape on bat movement has been almost  
100 exclusively described from visual observations (Arthur and Lemaire, 2015; Dietz et al., 2009). Some

101 studies on roads suggest that increasing distance to surrounding trees decreases bat crossing frequency,  
102 and that increasing tree height elevates bat crossing height (hence reducing the risk of collisions with  
103 vehicles) (Abbott, 2012; Bennett and Zurcher, 2013; Russell et al., 2009). But small sample sizes and  
104 poor taxonomic resolution limit the generalisation of these results. Moreover, Bennett and Zurcher  
105 (2013) considered bat trajectories initially directed perpendicular to the road and determined that  
106 vegetation structure and vehicle presence influenced bat decisions to cross the road or to fly away. But  
107 they did not take into account bats flying parallel to the road axis, although this behaviour may be a  
108 determinant factor of collisions, because in this situation bats spend much more time in the zone at  
109 collision risk.

110 Road collision risks in a species depend on (1) its local density, (2) the proportion of time spent in the  
111 zone at collision risk and (3) the simultaneous presence of bats and vehicles in the zone at collision risk  
112 (Jaeger et al., 2005; Zimmermann Teixeira et al., 2017). It is therefore necessary to take each of these  
113 conditional events into account when investigating road collisions. Indeed, when comparing two  
114 different road locations within different landscape features, a higher bat acoustic activity (used as a  
115 proxy of bat density) does not necessarily lead to a higher proportion of flights at collision risk for all  
116 species (see Abbott et al., 2012). In addition, even if more individuals are at risk of collision (or if  
117 mortality is higher) at one site compared to another, this does not necessarily mean that this site should  
118 be selected for mitigation. Indeed, local populations can be dramatically reduced due to road mortality  
119 year after year, and a measure of per capita mortality risk is essential to correctly identify dangerous  
120 locations and avoid wrong recommendations for the siting of mitigation measures (Zimmermann  
121 Teixeira et al., 2017). Per capita mortality is also a very useful tool to prioritise conservation actions in  
122 function of the susceptibility of species to anthropogenic impacts. For instance, bats of the *Nyctalus*  
123 genus are particularly susceptible to wind turbine collisions because a high proportion of the individuals  
124 are victims of collisions (Roemer et al., 2017a); to spare their populations, wind energy planning  
125 should therefore avoid areas where these species are extant.

126 Our study aimed at (1) assessing the effects of the local landscape on bat activity and movement  
127 behaviour and consequently on road collision risks, (2) disentangling the roles of density and movement

128 behaviour in collision risks, (3) determining how the orientation of linear vegetation affects the  
129 orientation of bat trajectories, to provide guidance for mitigation measures and (4) providing a proxy for  
130 species susceptibility to road collisions independently of their population sizes. In order to provide  
131 species-specific answers, our analyses were mostly performed at the species level, using the guild level  
132 only for species with small sample sizes. We used acoustic monitoring to detect bat passes and car  
133 passes, and acoustic flight path tracking to locate bat echolocation calls in three dimensions. This method  
134 allows reconstructing three-dimensional flight paths, and then model separately: (1) bat species density,  
135 (2) a probability of flight at collision risk, (3) a probability of bat-vehicle co-occurrence and (4) a  
136 probability to fly parallel or perpendicular to the road.

137 Concerning the response variables, we expected bat density to be the main factor influencing collision  
138 risks in some contexts (for example at tree rows along streams, which are rich in insects), but we  
139 expected the proportion of individuals flying in the zone at collision risks to be the main factor in other  
140 contexts (especially in forested areas, possibly forcing bats to fly over the road). Concerning explicative  
141 variables, we expected (1) a higher bat density at good quality habitats (i.e. tree rows near streams and  
142 tall trees) and at roads with a lower traffic rate, (2) a higher proportion of individuals in the zone at  
143 collision risk when vegetation grows closer to the road and in habitats with dense vegetation at each side  
144 of the road compared to habitats without trees, (3) a correlation between the orientation of bat trajectories  
145 and the orientation of linear vegetation, (4) a larger proportion of individuals flying in the zone at  
146 collision risk for short-range echolocators than for mid-range echolocators and long-range echolocators,  
147 reflecting the vertical niches of those species (Roemer et al., 2019).

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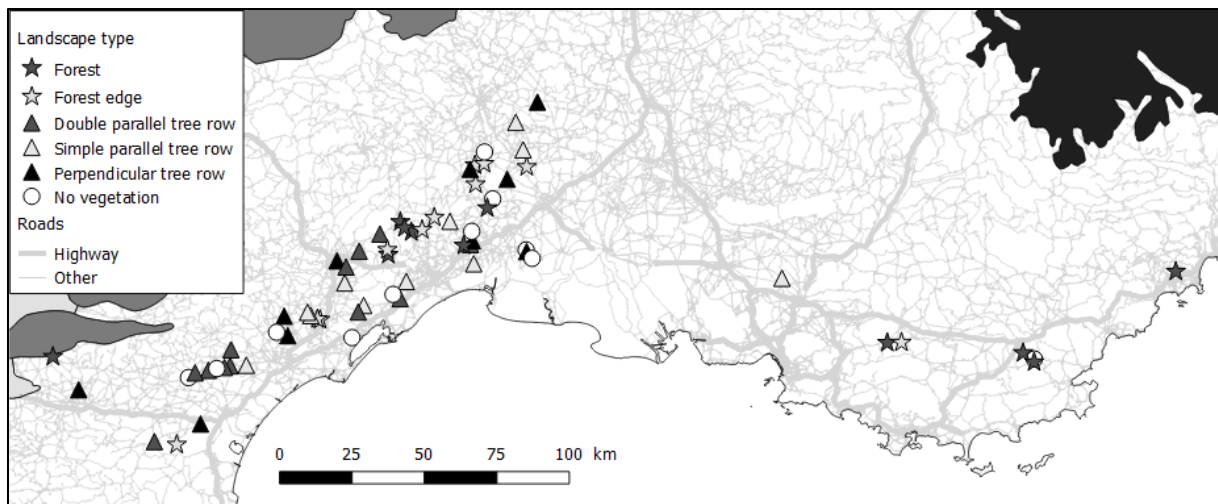
## 149 **Material and Methods**

### 150 *Study sites and description of the local landscape*

151 The study took place in 2016 and 2017 in the French Mediterranean lowland region. This area is  
152 composed of a mosaic of garrigues, cultivated areas (often vines), young forests of oaks and pines, and

153 urban areas consisting of small traditional villages and large cities with extensive conurbation. The  
154 national and departmental road network is built around one main highway going from the east to the  
155 west and following the French southern coastline (Figure 1). Four other highways link this main highway  
156 to the inner lands through large natural valleys.

157 Bat behaviour was recorded at 66 sites (Figure 1, supplementary table 1) at national or departmental  
158 roads, for a minimum of two nights per site, but recordings could continue up to five nights per site  
159 (mean = 2.6 nights +/- SD 0.9) depending on the schedule of the field worker and on battery strength.  
160 Sampling took place between the beginning of May and mid-October, depending on the study site. The  
161 local landscape was described within a radius of 30 m, equivalent to the sonar range of mid-range  
162 echolocating bats (Holderied and von Helversen, 2003). This scale was chosen under the assumption  
163 that bats adapt their flight movements according to the environment perceived acoustically. At most  
164 study sites, landscape description would have been similar with a 100 m radius, which corresponds to  
165 the sonar range of long-range echolocators (Holderied and von Helversen, 2003). Study sites were  
166 chosen so as to reach a balanced representation of six major types of road landscapes in the study area:  
167 simple parallel tree rows, double parallel tree rows, perpendicular tree rows, forests, forest edges and no  
168 vegetation taller than 1.5 m (Table 1, Figure 1, Figure A 1). Tree species were very often associated  
169 with a type of landscape: 70 % of simple and double parallel tree rows were plane trees (*Platanus sp.*),  
170 and more rarely olive trees (*Olea sp.*), Celtis (*Celtis sp.*), Aleppo pines (*Pinus halepensis*) or mulberries  
171 (*Morus sp.*). Forests and forest edges consisted in 80 % oaks (*Quercus ilex*, *Q. pubescens*, *Q. coccifera*  
172 and/or *Q. suber*), and 20 % Aleppo pines (*Pinus halepensis*). Perpendicular tree rows were a mix of  
173 Mediterranean riparian species (mostly *Fraxinus sp.*, *Populus sp.*, *Quercus sp.* and/or *Arundo donax*)  
174 typically associated with temporary watercourses. The category “no vegetation” consisted in land either  
175 occupied by vines, wheat, recently ploughed or left uncultivated. Pastureland was almost non-existent.  
176 Parallel tree rows had gaps of about 10 to 20 m between trees while the other types of vegetated  
177 landscapes had little or no gaps.



178

179 Figure 1: Geographical distribution of the 66 sampling sites in the secondary road network of the French  
 180 Mediterranean lowland region. The symbols of the sampling sites represent the type of local landscape  
 181 they belong to. Biogeographical regions are filled in colour: white: Mediterranean; grey: Continental;  
 182 black: Alpine. Road network source: ROUTE500 from the Institut National de l'Information  
 183 Géographique et Forestière (2017).  
 184

185 Table 1: Description of variables used to model bat density, bat position at collision risk, and flight path  
 186 orientation. Percentage of night, time lag and time lag direction were only used in the last two models.  
 187

<i>Variable</i>	<i>Description</i>	
<b><i>Landscape type</i></b>	<b><i>Simple parallel tree row</i></b>	One row of trees arranged linearly and parallel to the road.
	<b><i>Double parallel tree row</i></b>	Two rows of trees arranged linearly at each side and parallel to the road.
	<b><i>Perpendicular tree row</i></b>	One row of trees at each side and perpendicular to the road. Associated with small seasonal streams where water was either absent at the time of monitoring, or likely not accessible for bats due to dense tree cover.
	<b><i>Forest</i></b>	Dense tree patch at each side of the road.
	<b><i>Forest edge</i></b>	Dense tree patch at one side of the road.
	<b><i>No vegetation</i></b>	No vegetation taller than 1.5 m at each side of the road.
<b><i>Road width</i></b>	Distance between both outer edge lines of the roadway.	
<b><i>Traffic volume</i></b>	Mean number of vehicles per night.	
<b><i>Distance to tree foliage</i></b>	Mean distance between road outer edge line and tree foliage over all present trees. If foliage runs over the road, distance is negative.	
<b><i>Tree height</i></b>	Mean tree height from ground to canopy over all present trees.	

188

189 All sites were situated in lowlands, on two-lane asphalt roads of 4 to 8 m wide, on straight portions (at  
 190 least 200 m without curvature on each side of the sampling point), where vehicles were allowed to drive



191 up to 90 km/h. Several features were avoided: (1) artificial street lights and urban areas (the smallest  
192 distance to lit streets and urban areas was 300 m), (2) important three-dimensional structures, such as  
193 electric poles, (3) highways (the smallest distance to a highway was 1.1 km), (4) water bodies or  
194 wetlands other than the small streams sampled in the category “perpendicular tree rows” (the shortest  
195 distance to water was 100 m) and (5) sparse trees within the landscape matrix. The minimum distance  
196 between study sites was 500 m. Monitoring was performed exclusively during nights with optimal  
197 weather conditions for bat activity (temperature: mean = 20.6 +/- 6.5 °C, min = 8 °C, max = 34.9 °C;  
198 wind speed: mean = 7.5 +/- 8 km/h, min = 0 km/h, max = 31 km/h; cumulated rain per night: mean =  
199 0.2 +/- 1.3 mm, min = 0 mm, max = 11 mm). However, the percentage of visible moon (mean = 49.2  
200 +/- 35.8 %, min = 1 %, max = 99 %) was not a criterion we could control because of the time constrained  
201 field work schedule.

202 Four secondary landscape characteristics likely to affect flight behaviour were measured at each study  
203 site: road width, traffic volume, distance between road and tree foliage, and tree height (Table 1).  
204 Measurements were made with a laser telemeter. Traffic volume was calculated using the TADARIDA-  
205 L software (Bas et al., 2017) to identify and count vehicle passes. Sound event detection was done in  
206 the low frequency mode, and any acoustic sequence of 5 s or less that contained an uninterrupted sound  
207 event with a duration superior to 1.2 s was counted as one vehicle pass, even if several vehicles followed  
208 each other very closely. This threshold was chosen based on a verification of false and true positives  
209 and negatives on 100 random sound sequences from different study sites stratified by sound duration  
210 (unpublished data).

### 211 *Bat acoustic monitoring*

212 On each site, two pairs of microphones (either SMX-US or SMX-U1 (Wildlife Acoustics, USA), or  
213 BMX-US (Biotope, France)) were plugged into two SM2BATs or SM3BATs (Wildlife Acoustics,  
214 USA), each connected to a GPS unit used to timely synchronise recorders. Microphones were either  
215 mounted on wooden poles (at a maximum height of 4 m) or attached to vegetation (at a minimum height  
216 of 20 cm) (Figure A 1). Microphone pairs were installed on each side of the road (0.5 – 4 m distance

217 from the road edge) in arbitrarily shaped non-coplanar microphone arrays. Depending on the study site,  
218 minimum distance between microphones was 5.1 m and maximum distance was 22.6 m. Recorders were  
219 programmed to start each day 30 min before sunset and to stop 30 min after sunrise. Gain was set at 36  
220 dB for SMX-US and BMX-US microphones, or at 0 dB for SMX-U1 microphones. Sampling rate was  
221 set at 192 kHz, trigger at 6 dB above background noise and trigger window at 2.5 sec.

222 Species identification was performed based on echolocation calls, which carry enough information to  
223 allow the identification of the majority of European bat species, depending on the quality and the context  
224 of the recordings (Barataud, 2015). We used the SonoChiro software (Biotope/MNHN, France) to  
225 automatically sort sequences by species, and then verified most of the sequences manually on Syrinx  
226 (John Burt, USA) (except for sequences classified as *Pipistrellus* which are too numerous for a detailed  
227 verification, and because SonoChiro has a very low error rate for *P. pipistrellus* and *P. pygmaeus* in the  
228 Mediterranean region according to our experience). *Plecotus* species were grouped in *Plecotus* sp.,  
229 *Myotis blythii* and *M. myotis* were grouped in *M. blythii/myotis*, and *Pipistrellus kuhlii* and *P. nathusii*  
230 were grouped in *Pipistrellus kuhlii/nathusii*. Acoustic sequences that could not be identified to the  
231 species level or groups were left unidentified (0.15 % of all bat passes). From our knowledge of bat  
232 assemblages of France Mediterranean lowlands (unpublished mist-netting data), we expect the last  
233 group to contain a very large majority of *P. kuhlii*, and the *Plecotus* group to contain a very large majority  
234 of *P. austriacus*.

### 235 *Three-dimensional positioning of bat calls*

236 Bat three-dimensional flight paths were generated from the three-dimensional source location of  
237 echolocation calls recorded on the four microphones. After species acoustic identification, call location  
238 was achieved by (1) measuring time of arrival differences (TOAD) of each call between pairs of  
239 microphones and (2) deducing the coordinates of the sound source by comparing those field TOAD  
240 ( $TOAD_F$ ) with theoretical TOAD ( $TOAD_T$ ). Indeed, since the speed of sound in the air is known (here  
241 we approximated it to 340 m/s), TOADs of a sound source recorded by at least four microphones can  
242 be used to calculate the location of the source (see Koblitz, 2018).

243 TOAD<sub>F</sub> were calculated by measuring the starting time of bat calls using the SonoChiro software  
244 (Biotope/MNHN, France). Call association between pairs of microphones was achieved using the R (R  
245 Core Team, 2014) function `find.matches` of the Hmisc package (Harrell, 2018). Because there are four  
246 microphones, six TOAD<sub>F</sub> per call are calculated. TOAD<sub>T</sub> were calculated for each simulated point of a  
247 matrix of 40 x 40 x 40 m with a one-meter resolution and centred around the centroid of the 4  
248 microphones, inputting the same microphone configurations as the ones used in the field. The  
249 dimensions of this matrix were chosen according to the spatial range of our equipment (i.e. maximal  
250 distance of detection of a bat position) for the location of middle-range echolocators (e.g. *Pipistrellus*  
251 *pipistrellus*). This range is dependent on the acoustic range of the individual recorded and the position  
252 of the individual in relation to the microphones, i.e. accuracy is maximal at the centre of the device and  
253 minimal at the far edges.

254 The position of the bat was deduced from the comparison of the differences between the six TOAD<sub>F</sub> and  
255 the six TOAD<sub>T</sub> using the R (R Core Team, 2014) function `find.matches` of the Hmisc package (Harrell,  
256 2018). The closest TOAD match was selected as a candidate bat position. During test calibrations of our  
257 setting with different microphone configurations, we calculated that TOAD<sub>F</sub> resulting in a position more  
258 than 10 m away from the centroid of the microphones had a difference with the real position larger than  
259 one meter. Imprecise positions were systematically reconstructed away from the centroid, which means  
260 for example that a bat flying in reality at 15 m from the centroid could be located with our device at 17  
261 m (away from the centroid), but never at 13 m (toward the centroid). We therefore rejected any field  
262 position found at more than 10 m from the centroid of the microphones and did not use them for further  
263 analyses (Figure 2).

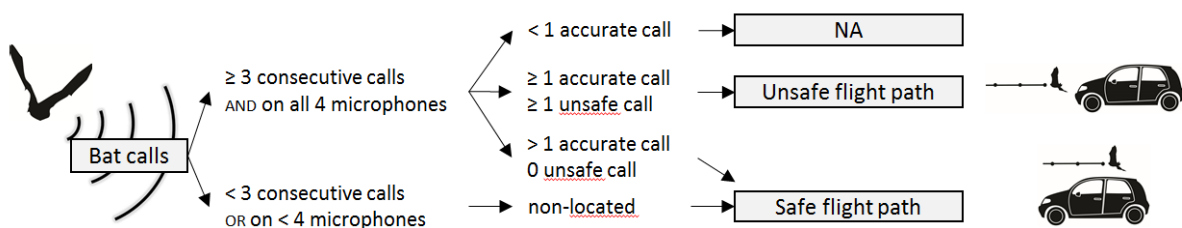
#### 264 *Grouping of calls in individual flight trajectories*

265 Calls were then attributed a flight trajectory ID using successive filters. During the first round, a same  
266 temporary ID was first given to all calls separated by less than two seconds and the flight speed between  
267 the preceding and the actual call was calculated in the X and Y dimensions. Several rounds were then  
268 run successively. At each round, to keep their temporary ID, calls had to (1) have a peak frequency

269 differing by less than 5 kHz from the median peak frequency of all calls within the same ID (2) be  
 270 separated by less than 2 seconds from the preceding call (3) be preceded and followed by positions  
 271 conferring a speed lower than 20 m/s (i.e. the maximum possible speed of flying bats (Holderied and  
 272 Jones, 2009; Popa-Lisseanu, 2007). Otherwise, calls were attributed a new (unique) temporary ID and  
 273 went through a new round of filtering. Successive rounds were applied until all IDs were stabilized.  
 274 Flight trajectories with less than three calls were not considered as a full flight path and were classified  
 275 as non-located bat passes (Figure 2). R scripts and tables are available at [https://github.com/Charlotte-](https://github.com/Charlotte-Roemer/bat-road-collision-risks)  
 276 [Roemer/bat-road-collision-risks](https://github.com/Charlotte-Roemer/bat-road-collision-risks).

277 *Definition of collision risk and calculation of flight path orientation*

278 Each successfully located bat position above the road and at vehicle height (< 5 m) (Berthinussen and  
 279 Altringham, 2012) was classified as ‘unsafe’ (Figure 2). All other successfully located positions were  
 280 classified as ‘safe’. Bat calls which were not recorded by all four microphones at once – and that could  
 281 therefore not be precisely located – were assumed to be far from the microphones’ centroid and thus  
 282 probably far from the road and hence also classified as ‘safe’. For the same reason, positions potentially  
 283 not successfully located (> 10 m from microphones centroid) were disregarded to avoid location errors.  
 284 Since this error rate is similar across landscape types, we do not expect any resulting bias. If any of the  
 285 bat positions within a flight trajectory was unsafe, the complete flight trajectory was classified as unsafe,  
 286 otherwise it was classified as safe. Flight trajectories with less than three calls were assumed to be far  
 287 from the microphones’ centroid and thus probably far from the road and hence also classified as ‘safe’  
 288 (Figure 2).



289

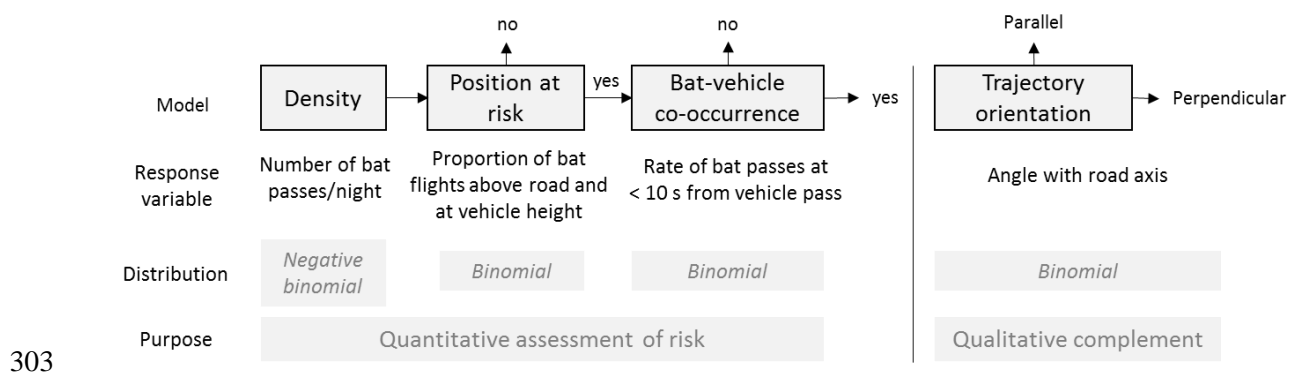
290 Figure 2: Process of classification of flight trajectory at collision risk. NA: non-available data. The area  
 291 where calls are accurate is 10 m around the centroid of microphones. Unsafe call: located at vehicle  
 292 height and above the road.

293

294 We then calculated the angle between the road axis and the axis of the vector linking the first to the last  
295 position of each flight paths. Trajectories were classified in two categories:  $0-45^\circ$  = parallel;  $45-90^\circ$  =  
296 perpendicular to the road.

### 297 *Response variables*

298 We tested how the local landscape affects the different determinants of collision risks, building one  
299 model for each of them: (1) local species density (2) the proportion of flights in the zone at risk (3) bat-  
300 vehicle co-occurrence and (4) flight path orientations (Figure 3). To summarise the results, the estimates  
301 of the first three models (i.e. quantitative models) were multiplied to obtain as a product (5) the number  
302 of bat passes at collision risk per night, depending on the characteristics of the local landscape.



303

304 Figure 3: Successive steps in modelling of bat density and flight behaviour in function of landscape  
305 variables. If the flight path is at risk, position at risk = yes. If the time lag between the bat pass and the  
306 vehicle pass is inferior to 10 s, bat-vehicle co-occurrence = yes.  
307

#### 308 (1) Local bat density

309 The density of the most common species in our dataset (i.e. occurrence per night larger than 30 % and  
310 occurrence per site larger than 50 %) was modelled as a negative binomial distribution. The response  
311 variable was the median number (among the four microphones) of five second intervals per night within  
312 which a species was identified. This acoustic activity was then used as a proxy of bat density (number  
313 of acoustic sequences per night within the acoustic range of the setting) (Froidevaux et al., 2017).

314 (2) Probability of bats to fly through the zone at risk

315 The probability of trajectories to be in the zone at risk (i.e. at vehicle height) was modelled using the  
316 risk status of each trajectory as the binomial response variable (0 = safe; 1 = unsafe) (Figure 2).

317 (3) Bat-vehicle co-occurrence

318 The probability of bats flying through the zone at risk gives a spatial evaluation of risk. To make a more  
319 precise risk assessment, bat-vehicle co-occurrence (i.e. temporal evaluation of risk) was also modelled.  
320 For bat flight trajectories at risk only, a proxy for the probability of bats avoiding vehicles was modelled  
321 using bat-vehicle co-occurrence as the binomial response variable (1 = bat-vehicle co-occurrence; 0 =  
322 presence of a bat while absence of vehicle). To do this, the time lag between an acoustic sequence  
323 containing a bat and the closest sequence containing a vehicle pass was calculated using the function  
324 `find.matches` of the `Hmisc` package (Harrell, 2018). If the time lag was lower than 10 s, we considered  
325 that there was a bat-vehicle co-occurrence (1). If the time lag was higher than 10 s, we considered that  
326 a bat was present during the absence of a vehicle (0). A relatively higher proportion of long time lags  
327 between bat passes and vehicle passes could be interpreted as a higher probability that bats avoid  
328 vehicles, thus we used this metric as a proxy for vehicle avoidance.

329 (4) Flight path orientation

330 For all bat flight trajectories, the proportion of flight paths parallel to the road axis was modelled using  
331 flight orientation as a binomial response variable (0 = perpendicular; 1 = parallel).

332 (5) Number of bat passes at collision risk per night

333 If an explanatory variable was selected in several models, then each of those models gives a partial  
334 evaluation of bat collision risks on roads. In fact, all quantitative models succeeding the density model  
335 can be interpreted as conditional probabilities that an individual is at risk of collision. Thus, the number  
336 of bat passes at risk of collision on a road section can be computed by the multiplication of all outputs  
337 of the quantitative models:

338 Eqn 1: *Expected value of collision risk* =  $E_1 \times E_2 \times E_3$ ,

339 where:

340  $E_1$  = Prediction of the number of bats present on site per night

341  $E_2$  = Prediction of the probability that a detected bat flies in the volume at collision risk

342  $E_3$  = Prediction of the probability that a bat in the volume at risk co-occurs with a vehicle pass

343 To estimate the confidence intervals of this product, we needed a large number of responses for each  
344 value along the gradient of each predictor for each response variable. For this, we first simulated  
345 responses according to model estimates and their standard error using the `rnorm` function (R Core Team,  
346 2014): 20,000 replicates for each of 60 values along a gradient for a given predictor. For a given  
347 predictor, if one  $E_1$ ,  $E_2$  or  $E_3$  was missing, meaning that the predictor was not selected in one of the  
348 models, we used the mean value of the original observations instead. If two or all three of  $E_1$ ,  $E_2$  or  $E_3$   
349 were missing for a given predictor, we did not compute their product.

350 Since our results apply for road sections 20 m in length, we multiplied the expected mean number of bat  
351 passes at collision risk by 50 to obtain a mean number of bat passes at risk of collision per kilometre  
352 and per night. To compare bat guilds susceptibility to road collisions, we multiplied  $E_2 \times E_3$ ; this result  
353 is an index of susceptibility to road collisions that is independent of local population densities.

#### 354 *Model selection*

355 We used the R (R Core Team, 2014) package `glmmTMB` (Brooks et al., 2017) to model each response  
356 variable in generalised linear mixed models (GLMM). When sample size of a given species was too  
357 small, we did not model the species response. In addition, three bat guilds were created based on the  
358 adaptation of species to clutter of the environment, which is strongly linked to sonar features (Aldridge  
359 and Rautenbach, 1987; Denzinger et al., 2018). Species were thereby split into the guilds “short-range  
360 echolocator” (SRE), “mid-range echolocator” (MRE) or “long-range echolocator” (LRE) according to  
361 the definition of Frey-Ehrenbold et al. (2013) (see Table A 1 for complete list).

362 All descriptive variables were normalised if necessary and scaled to follow a normal distribution and to  
363 compare their effects. Variables considered for fixed effects were landscape type, road width, traffic  
364 volume, distance to tree foliage and tree height (Table 1 **Erreur ! Source du renvoi introuvable.**). We  
365 first calculated the correlation coefficients between predictors using the corrplot function of the stats  
366 package in the R program (R Core Team, 2014). Tree height and distance to tree foliage were correlated  
367 ( $r = -0.57$ ), as well as road width and traffic volume ( $r = 0.64$ ). We excluded road width for further  
368 analysis and created the possibility to select either tree height or distance to tree foliage (but not both)  
369 during stepwise model selection (see next paragraph). Candidate predictors were also included in simple  
370 interactions with each other. In addition, Julian day was included as a fixed quadratic effect to account  
371 for seasonal variations in bat density and flight behaviour. Site ID was introduced as a random effect.

372 An upward stepwise model selection was performed to select the relevant variables (except for Julian  
373 day which was part of the null model). We operated an upward model selection because the full model  
374 led to overfitting for species with a low occurrence. At each step of model selection, the VIF (Variance  
375 inflation factor), which quantifies the degree of multicollinearity in least square regression analyses, was  
376 calculated. If any of the selected variables had a  $VIF > 3$  (Heiberger and Holland, 2004; Zuur et al.,  
377 2010), the model was not considered as a candidate model. At each step of model selection, the model  
378 with the smallest Akaike's information criterion for small sample sizes (AICc) was considered. This  
379 model was retained and selected if its AICc was at least inferior by two points to the AICc of the best  
380 model of the previous step (supporting that the newly added parameter is informative) (Arnold, 2010).

381 For each retained model, we checked the uniformity of the residuals using the DHARMA package  
382 (Hartig, 2018). Goodness of fit, autocorrelation, overdispersion and zero-inflation (for density data only)  
383 were checked and revealed no problematic situation.

384

385 **Results**



386 In total, 122,294 bat passes were recorded and identified at the group or species level, from which 30,954  
387 successful flight trajectories could be located (Table A 1). Because of technical problems on two study  
388 sites (one of the two recorders was once destroyed by a rotary flail and once displaced by someone),  
389 flight path tracking could not be carried out and these sites were used for modelling bat density only.  
390 The density of nine species and three species groups (*Pipistrellus kuhlii/nathusii*, *Plecotus sp.* and  
391 *Myotis blythii/myotis*) could be modelled, but their flight behaviour (i.e. presence at risk, bat-vehicle co-  
392 occurrence and flight path orientation) could not be modelled for all of the species or species groups,  
393 due to the lack of data.

394 Models showed no convergence problem during selection, except for *E. serotinus* (model position in the  
395 zone at risk, for interactions), *M. daubentonii* (model bat-vehicle co-occurrence, for landscape type), *H.*  
396 *savii* (model trajectory orientation, for landscape type) and *M.myotis/blythii* (model bat-vehicle co-  
397 occurrence, for landscape type; model trajectory orientation, for landscape type).

398

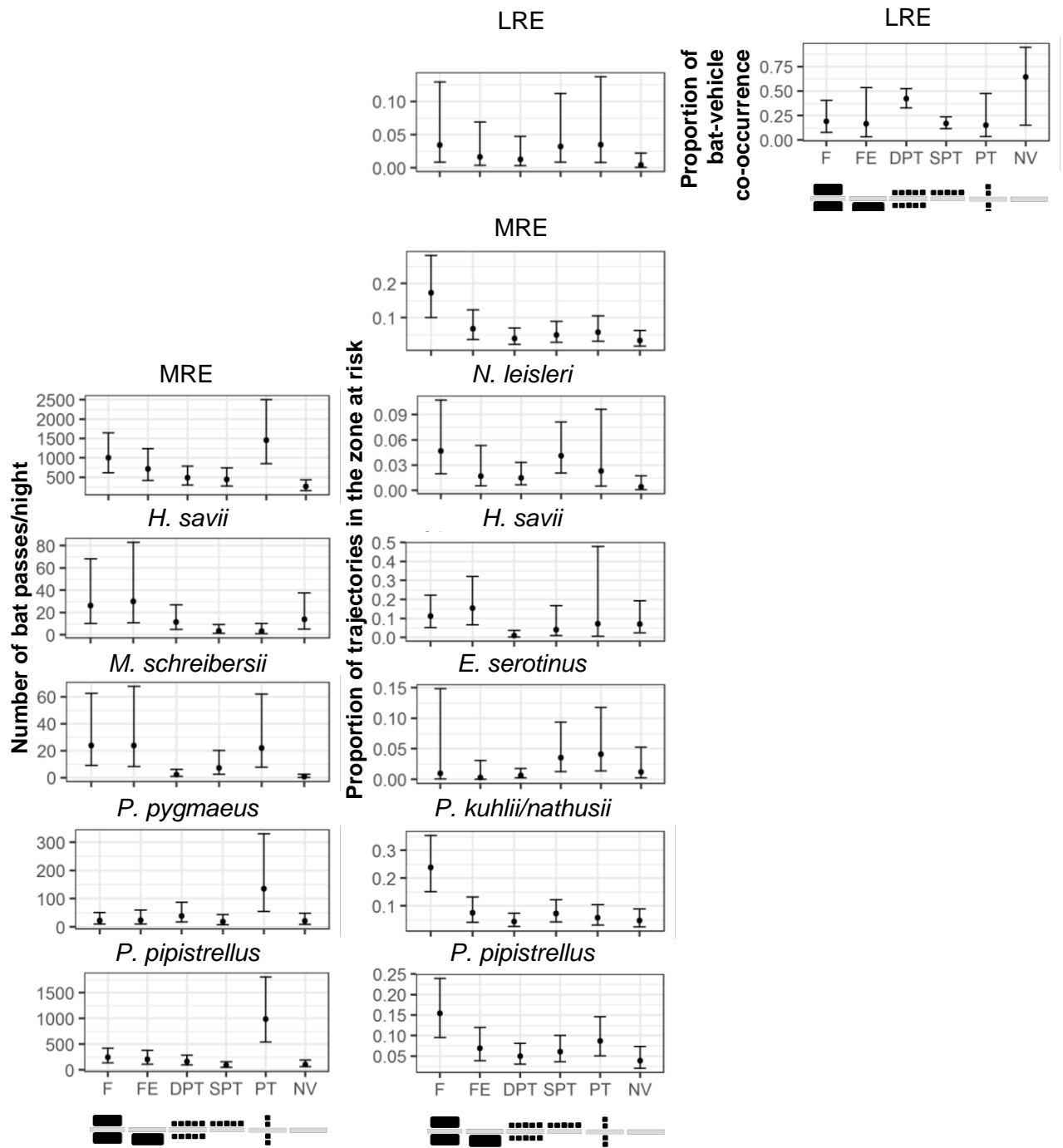
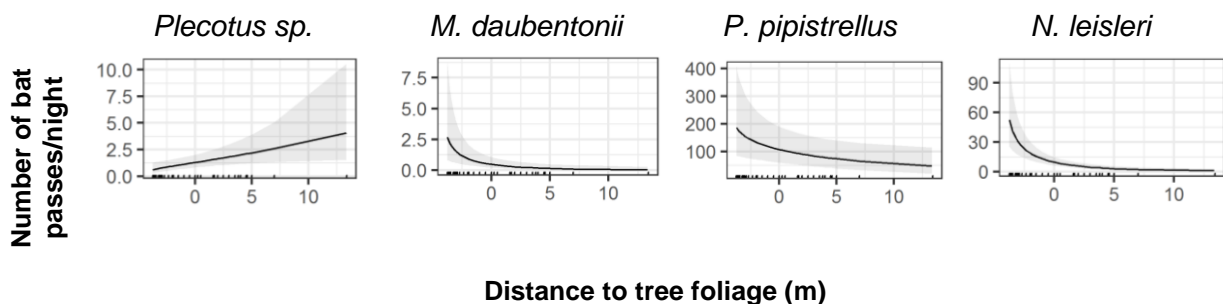


Figure 4: Predicted effects of landscape type on density (left), proportion of trajectories at risk (middle) and predicted proportion of bat-vehicle co-occurrence (trajectories at risk and at less than 10 s from a vehicle pass) (right). 95% confidence intervals are shown. Only the effects present in the final models are shown. Bottom figures represent landscape type viewed from the top (road in light grey and trees in black). LRE: long-range echolocators. MRE: mid-range echolocators. F = forest. DPT = double parallel tree rows. NV = no vegetation. FE = forest Edge. PT = perpendicular tree rows. SPT = simple parallel tree rows.

400 *Model 1 – Bat density*

401 Landscape type had an important influence on bat density (Table 2). It was selected in the model of four  
402 species. Density was much higher at perpendicular tree rows for *Pipistrellus* species and was higher at  
403 forested landscapes for *H. savii* (Figure 4). Increasing distances to tree foliage were associated with a  
404 decrease in bat density for five species (*M. daubentonii*, *P. pipistrellus*, *P. pygmaeus*, *M. schreibersii*  
405 and *N. leisleri*) and for the MRE guild, while it was associated with an increase for *Plecotus* species  
406 (Figure 5). Increasing tree height was associated with an increase in the density of *M. blythii/myotis* and  
407 of the LRE guild (Table 2). With an increasing traffic volume, the density of *Plecotus sp.* and of the  
408 SRE guild decreased (Table 2). Throughout the year, species density showed a typical peak in mid-  
409 summer, except for *P. pygmaeus*, *M. schreibersii*, *Plecotus sp.* and *N. leisleri*, that were more active in  
410 the autumn (Figure A 2).

411



412  
413 Figure 5: Predicted effect of distance to foliage on bat density for a selection of four species. 95%  
414 confidence intervals are shown. Ticks in x axis represent sampled values. Negative values mean that  
415 foliage was running over the road.  
416

417 *Model 2 – Bat presence in the zone at risk*

418 Landscape type also greatly influenced the proportion of bat positions in the zone at risk. It was selected  
419 in five of the ten species-specific models, and in two of the guild models. The proportion of positions at  
420 risk was generally higher in forests and lower without trees (Figure 4 and Table 3).

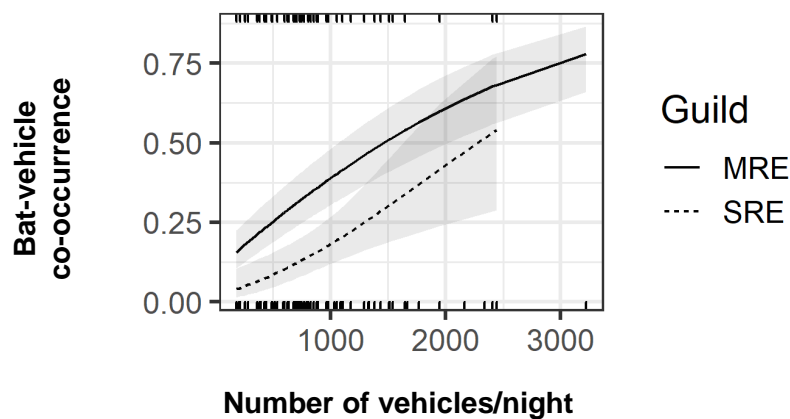
421 An increasing distance to tree foliage was associated with an increase in the presence at risk for *E.*  
422 *serotinus* (Table 3). Increasing traffic density was associated with a decrease in the proportion of flights

423 in the zone at risk for *P. kuhlii/nathusii* (Table 3). Throughout the year, the different species displayed  
424 quite different patterns in presence at risk, but all three guilds showed a tendency for a higher proportion  
425 of flights in the zone at risk toward the end of the year (Figure A 2).

#### 426 *Model 3 – Bat-vehicle co-occurrence for trajectories in the zone at risk*

427 Landscape type was only selected in the model for the LRE (Table 4). For this guild, double parallel  
428 tree rows were associated with higher bat-vehicle co-occurrence than simple parallel tree rows.  
429 Increasing tree height was associated with an increase in bat-vehicle co-occurrence in *M. daubentonii*,  
430 *P. pipistrellus*, *P. pygmaeus* and the MRE guild (Table 4). An increasing traffic density was associated  
431 to an increase in bat-vehicle co-occurrence for all *Pipistrellus* species and for *M. myotis/blythii* (Table  
432 4). The MRE guild had a higher rate of bat-vehicle co-occurrence than the SRE guild (Figure 6). Season  
433 had different effects on bat-vehicle co-occurrence according to species and guilds (Table 4, Figure A 2).

434



435 Figure 6: Predicted effect of traffic volume on proportion of bat-vehicle co-occurrence (trajectories  
436 positioned at risk and at more than 10 s from a vehicle pass). 95% confidence intervals are shown. Ticks  
437 in x axis represent sampled values (bottom = MRE; top = SRE). SRE: short-range echolocators. MRE:  
438 mid-range echolocators.  
439  
440

#### 441 *Model 4 – Orientation of flight trajectories*

442 The large majority of flight paths followed the road axis in all landscape types (results not shown).  
443 Landscape type, distance to tree foliage, and tree height were not selected to explain trajectory  
444 orientation (Table 5). Nonetheless, an increasing traffic volume was associated to a larger proportion of

445 trajectories parallel to the road axis in *P. pipistrellus* (Table 5). Season had a very weak, or even no  
446 effect on the proportion of trajectories parallel to the road (Figure A 2).

447 *Product: number of bat passes at collision risk per night*

448 There was only a small selection of species for which the same variable had an effect on at least two  
449 aspects of the collision risk (i.e. on bat density, bat presence in the zone a risk, or bat-vehicle co-  
450 occurrence). These cases are all described in this section. An increase in traffic was associated with a  
451 tendency of an increasing number of bat passes at risk of collision for *P. kuhlii/nathusii* and the SRE  
452 guild (Figure A 3). The number of bat passes at collision risk was higher at perpendicular tree rows for  
453 *P. pipistrellus* but higher at forests and forest edges for *H. savii* (Figure A 3). The number of bat passes  
454 at risk of collision was higher in summer for *E. serotinus*, *H. savii*, *P. kuhlii/nathusii* and *P. pipistrellus*,  
455 while it was higher in autumn for *M. schreibersii*, *M. daubentonii*, *P. pygmaeus* and *Plecotus sp.*, and  
456 higher in spring for *M. myotis/blythii* (Figure A 2).

457 We found a mean number of bat passes at risk of collision per kilometre and per night of 2.3 for SRE,  
458 1024.9 for MRE and 11.7 for LRE (Figure 7).

459 The index of susceptibility to road collisions, which is independent of species population densities,  
460 placed MRE as the most susceptible guild (Figure 8).

461

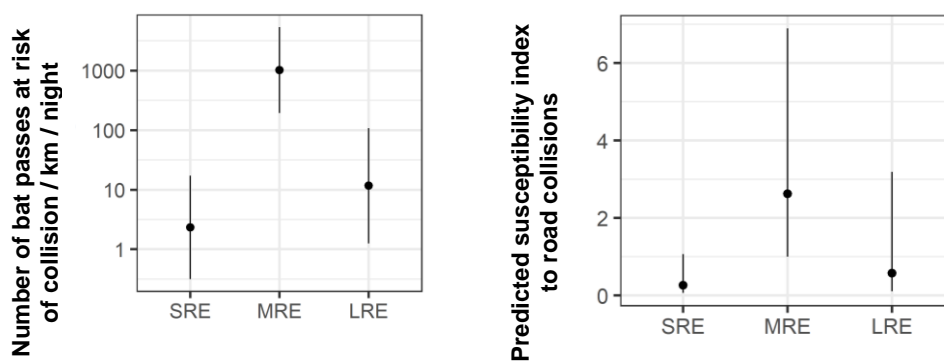


Figure 7: Predicted number of bat passes at risk of collision per night and per kilometre for each bat guild (logarithmic scale). 95% confidence intervals are shown. SRE: short-range echolocators. MRE: mid-range echolocators. LRE: Long-range echolocators.

Figure 8: Predicted susceptibility index to road collisions for each bat guild. 95% confidence intervals are shown. SRE: short-range echolocators. MRE: mid-range echolocators. LRE: Long-range echolocators.

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Table 2: Summarised statistical results of the negative binomial distributed GLMM for the density of each species.  $\hat{\beta}$  = estimate. SE = standard error. p = significance of p value. Species names are given with the three first letters of the species and genera. Dist.tree = distance to tree foliage. Tree.H = tree height. DPT = double parallel tree rows. F = forest. FE = forest edge. PT = perpendicular tree rows. SPT = simple parallel tree rows. Intercept is for NV (no vegetation) landscape type. LRE: long-range echolocators. MRE: mid-range echolocators. SRE: short-range echolocators. There is a total of 163 nights of observations.

Variable Type	Eptser			Hypsav			Minsch			Myodau			Myoema			Myobly/myo			Nyclei			Pipkuh/nat			Pippip			Pippyg			Plesp		
	$\hat{\beta}$	SE	p	$\hat{\beta}$	SE	p	$\hat{\beta}$	SE	p	$\hat{\beta}$	SE	p	$\hat{\beta}$	SE	p	$\hat{\beta}$	SE	p	$\hat{\beta}$	SE	p	$\hat{\beta}$	SE	p	$\hat{\beta}$	SE	p	$\hat{\beta}$	SE	p			
<b>Intercept</b>	0.64	0.48		2.64	0.50	***	-0.17	0.56		-0.76	0.41	.	0.00	0.28		0.32	0.39		2.24	0.22	***	5.50	0.19	***	4.67	0.29	***	3.03	0.44	***	0.26	0.23	
<b>Dist.tree</b>							-0.58	0.23	*	-0.92	0.29	**							-0.91	0.17	***				-0.29	0.13	*	-0.80	0.20	***	0.42	0.16	*
<b>Tree.H</b>																1.21	0.33	***															
<b>Traffic</b>																															-0.44	0.17	*
<b>DPT</b>				-0.20	0.62		1.04	0.72																0.44	0.39		0.62	0.59					
<b>F</b>				0.63	0.64		3.34	0.71	***														0.82	0.38	*	0.06	0.58						
<b>FE</b>				0.76	0.63		3.34	0.72	***														0.67	0.39	.	0.14	0.59						
<b>PT</b>				-1.42	0.67	*	3.26	0.72	***														2.22	0.39	***	1.87	0.59	**					
<b>SPT</b>				-1.35	0.67	*	2.15	0.75	**														-0.16	0.40		-0.11	0.61						
<b>Julian Day</b>	-0.19	0.30		-0.21	0.18		0.42	0.15	**	1.17	0.32	***	-0.30	0.18		-0.85	0.28	**	0.40	0.14	**	-0.09	0.13		0.40	0.10	***	0.74	0.14	***	0.53	0.15	***
<b>Julian Day<sup>2</sup></b>	-0.96	0.30	**	-0.99	0.19	***	0.36	0.14	**	-0.58	0.26	*	-0.44	0.19	*	-0.75	0.23	**	0.28	0.14	*	-0.50	0.12	***	-0.58	0.09	***	0.07	0.13		0.41	0.14	**

P < 0.1 = .  
P < 0.05 = \*  
P < 0.01 = \*\*  
P < 0.001 = \*\*\*

468

469 Table 2 (continued)

Variable Type	SRE			MRE			LRE		
	$\hat{\beta}$	SE	p	$\hat{\beta}$	SE	p	$\hat{\beta}$	SE	p
<b>Intercept</b>	3.09	0.16	***	5.56	0.26	***	3.17	0.19	***
<b>Dist.tree</b>				-0.31	0.12	**			
<b>Tree.H</b>							0.71	0.15	***
<b>Traffic</b>	-0.36	0.14	**						
<b>DPT</b>				0.62	0.35	.			
<b>F</b>				1.35	0.34	***			
<b>FE</b>				1.01	0.35	**			
<b>PT</b>				1.72	0.35	***			

Variable	SRE			MRE			LRE		
	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p
<b>SPT</b>				0.53	0.36				
<b>Julian Day</b>	0.19	0.10	.	0.18	0.09	*	0.30	0.13	*
<b>Julian Day<sup>2</sup></b>	-0.29	0.10	**	-0.30	0.08	***	-0.05	0.12	

470 P < 0.1 = .  
471 P < 0.05 = \*  
472 P < 0.01 = \*\*  
473 P < 0.001 = \*\*\*

474 Table 3: Summarised statistical results of the binomial distributed GLMM for the proportion of trajectories in the zone at risk for each species.  $\beta$  = estimate. SE = standard error.  
475 p = significance of p value. Species names are given with the three first letters of the species and genera. Dist.tree = distance to tree foliage. Tree.H = tree height. DPT = double  
476 parallel tree rows. NV = no vegetation. FE = forest Edge. PT = perpendicular tree rows. SPT = simple parallel tree rows. Intercept is for F (forest) landscape type. LRE: long-  
477 range echolocators. MRE: mid-range echolocators. SRE: short-range echolocators.  
478

Variable	Eptser			Hypsav			Minsch			Myodau			Myobly/myo			Nyplei			Pipkuh/nat			Pippip			Pippyg			Plesp			SRE			MRE			LRE		
<b>N observations</b>	876			4509			10280			401			1629			8401			45475			40279			19446			743			5744			119991			10025		
Type	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p			
<b>Intercept</b>	-4.77	1.5	**	-2.53	0.46	***	-3.6	0.31	***	-1.95	0.18	***	-3.7	0.42	***	-3.21	0.46	***	-1.2	0.29	***	-1.7	0.28	***	-2.91	0.17	***	-3.69	0.54	***	-3.51	0.36	***	-1.55	0.32	***	-3.48	0.73	***
<b>Dist.tree</b>	1.09	0.31	***																																				
<b>Tree.H</b>																																							
<b>Traffic</b>																																							
<b>DPT</b>	-0.4	1.31		-2.57	0.79	**																																	
<b>FE</b>	-1.31	1.43		0.38	0.59																																		
<b>NV</b>	0.18	1.46		-0.51	0.67																																		
<b>PT</b>	1.46	1.4		-0.49	1.28																																		
<b>SPT</b>	1.29	1.59		-1.09	0.84																																		
<b>Julian Day</b>	0.03	0.34		-1.39	0.53	**	0.43	0.21	*	0.25	0.26		0.59	0.28	*	0.38	0.2	.	0.01	0.1		-0.01	0.08		0.23	0.13	.	0.48	0.33		0.38	0.15	*	0.11	0.06	*	0.24	0.17	
<b>Julian Day<sup>2</sup></b>	1.61	0.48	***	-1	0.65		-0.37	0.15	*	-0.14	0.21		0.45	0.24	.	0	0.19		0.04	0.09		-0.09	0.09		-0.04	0.12		-0.11	0.3		-0.1	0.14		-0.05	0.06		0.13	0.17	

479 p < 0.1 = .  
480 p < 0.05 = \*  
481 p < 0.01 = \*\*  
482 p < 0.001 = \*\*\*

483 Table 4: Summarised statistical results of the binomial distributed generalised linear mixed effect models (GLMM) for bat-vehicle co-occurrence for each species.  $\beta$  = estimate.  
484 SE = standard error. p = significance of p value. Species names are given with the three first letters of the species and genera. Dist.tree = distance to tree foliage. Tree.H = tree  
485 height. DPT = double parallel tree rows. NV = no vegetation. FE = forest Edge. PT = perpendicular tree rows. SPT = simple parallel tree rows. Intercept is for F (Forest)  
486 landscape type. LRE: long-range echolocators. MRE: mid-range echolocators. SRE: short-range echolocators.



Variable	Eptser			Hypsav			Minsch			Myodau			Myobly/myo			Nyclei			Pipkuh/nat			Pippip			Pippyg			SRE			MRE			LRE								
N observations	35			869			661			46			60			432			5155			5291			1356			213			13332			480								
Type	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p						
Intercept	0.23	0.63		0.25	0.27		-1.32	0.30	***	-1.83	0.60	**	-0.63	0.81		-0.41	0.34		-0.66	0.09	***	-0.69	0.12	***	-0.89	0.14	***	-1.56	0.25	***	-0.51	0.19	**	-0.43	0.44							
Dist.tree																																					0.57	0.21	**			
Tree.H										0.72	0.31	*										0.18	0.09	*	0.32	0.09	***				0.15	0.07	*									
Traffic													2.79	1.81								0.79	0.09	***	0.60	0.10	***	0.53	0.12	***	0.89	0.25	***	0.65	0.08	***						
DPT																																					1.12	0.52	*			
FE																																					-0.18	0.80				
NV																																					2.03	1.18	.			
PT																																					-0.27	0.72				
SPT																																					-0.16	0.55				
Julian Day	0.74	0.53		-0.40	0.59		0.49	0.19	**	-0.46	0.58		-1.00	0.48	*	-0.45	0.30		-0.01	0.10		0.20	0.10	.	0.05	0.12		-0.45	0.21	*	0.09	0.08		-0.16	0.25							
Julian Day <sup>2</sup>	0.10	0.74		-1.00	0.58	.	0.37	0.17	*	0.49	0.45		-0.10	0.38		-0.16	0.24		0.07	0.09		-0.11	0.10		0.08	0.11		0.23	0.18		0.02	0.07		-0.10	0.19							

p < 0.1 = .  
 p < 0.05 = \*  
 p < 0.01 = \*\*  
 p < 0.001 = \*\*\*

489 Table 5: Summarised statistical results of the binomial distributed generalised linear mixed effect models (GLMM) for the flight path orientation for each species.  $\beta$  = estimate.  
 490 SE = standard error. p = significance of p value. Species names are given with the three first letters of the species and genera. Dist.tree = distance to tree foliage. LRE: long-  
 491 range echolocators. MRE: mid-range echolocators. SRE: short-range echolocators.  
 492

Variable	Eptser			Hypsav			Minsch			Myobly/myo			Nyclei			Pipkuh/nat			Pippip			Pippyg			Plesp			SRE			MRE			LRE								
N observations	97			1956			1309			196			657			10787			10440			2834			81			625			27236			625								
Type	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p	$\beta$	SE	p						
Intercept	2.32	0.48	***	1.00	0.38	**	1.08	0.21	***	1.53	0.33	***	1.19	0.34	***	1.42	0.11	***	1.38	0.12	***	1.56	0.17	***	1.41	0.65	*	1.77	0.29	***	1.51	0.12	***	1.55	0.30	***						
Traffic																			0.24	0.10	*																					
Julian Day	0.68	0.47		0.65	0.77		0.22	0.14		0.06	0.18		-0.01	0.26		0.02	0.10		-0.17	0.09	.	0.22	0.12	.	0.31	0.32		0.36	0.20	.	0.06	0.07		0.20	0.23							
Julian Day <sup>2</sup>	-0.66	0.51		1.38	1.09		0.02	0.13		-0.28	0.20		0.02	0.22		-0.12	0.09		-0.05	0.09		-0.06	0.12		-0.27	0.35		-0.35	0.20	.	-0.14	0.07	*	-0.18	0.21							

P < 0.1 = .  
 P < 0.05 = \*  
 P < 0.01 = \*\*  
 P < 0.001 = \*\*\*

## 494 Discussion

495 This study aimed at disentangling the different mechanisms that influence bat-vehicle collision risks:  
496 (1) density of individuals recorded from the road edge, (2) position in the zone at risk (low flight height  
497 over the road), (3) bat-vehicle co-occurrence, and (4) flight path orientation. Disentangling those  
498 mechanisms was possible thanks to three-dimensional acoustic flight path tracking of bat behaviour at  
499 roads on a large scale (French Mediterranean region) on a large amount of data (122,294 bat passes).  
500 Our results demonstrate heterogeneity in the influence of explanative variables on the four response  
501 variables, depending on species.

### 502 *Local landscape type*

503 The effect of local landscape type on density was heterogeneous according to species. For example,  
504 perpendicular tree rows led to a higher density of *P. pygmaeus* compared to simple parallel tree rows,  
505 and forest edges led to a higher density of *H. savii* compared to simple parallel tree rows. It is rather  
506 unlikely that the structural composition (density and orientation of linear vegetation) is the only  
507 explanation for these differences, because other confounding effects may very well increase bat density  
508 also, such as the different tree species that were often associated with a type of landscape.

509 Interestingly, landscape type did not produce similar effects on bat density and on bat flight behaviour.  
510 Indeed, in the case of *P. pipistrellus* for instance, bat density was the highest in perpendicular tree rows  
511 formed by small streams. However, local landscapes eliciting the highest proportion of flights at  
512 collision risk for this species were forests. This type of landscape was in fact a very high factor of  
513 presence at risk for most species and guilds, as we expected. Vegetation density may play an important  
514 role here. Indeed, parallel tree rows consisted in individual planted trees with gaps of 10 to 20 m while  
515 forests had dense shrub layers most of the time. Tree rows probably allow bats to benefit from the edge  
516 effect (i.e. easy access to flying insects abundant in or near tree foliage) (Brigham et al., 1997; Evans et  
517 al., 2003; Verboom and Spoelstra, 1999) without needing to fly directly above the road, contrarily to  
518 forest landscapes with hard edges, which act as conduits (Kalcounis-Rueppell et al., 2013). In addition,  
519 for the LRE guild, results show that bat-vehicle co-occurrence was higher at double parallel tree rows

520 compared to simple parallel tree rows. It seems difficult to provide a straight-forward explanation here,  
521 but the higher social activity of *N. leisleri* that we recorded at double parallel tree rows could be linked  
522 to less vehicle avoidance.

523 Contrary to our expectations, landscape types were not selected to explain the orientation of flight  
524 trajectories. Our results show that even in the presence of a perpendicular tree row or in the absence of  
525 trees, bats fly most of the time parallel to the road axis. This supports the idea, not often enough  
526 emphasised in collision risk assessments, that bats may use roads as corridors, because road verges may  
527 offer foraging opportunities by attracting more insects than adjacent habitats (Medinas et al., 2019;  
528 Villemey et al., 2018), and because of the verge effect when trees are present (Brigham et al., 1997;  
529 Kalcounis-Rueppell et al., 2013; Verboom and Spoelstra, 1999). According to our results, it should be  
530 considered that on secondary roads, bats following the road axis may be as common as bats crossing  
531 roads, and that mitigation measures should deal with these two types of movements.

#### 532 *Tree height and distance to tree foliage*

533 As said in material and methods, distance to tree foliage was correlated with tree height in our study. In  
534 all species except *Plecotus sp.*, density was negatively affected by an increasing distance to tree foliage,  
535 when selected. Conversely, taller trees led to a higher density of *M. myotis/blythii* and of the LRE guild.  
536 Our hypothesis according to which taller trees would be associated with a higher density was thus only  
537 verified for one species. The effect of distance to trees was shown in several studies out of road context  
538 (Heim et al., 2015; Kelm et al., 2014; Verboom and Spoelstra, 1999), and once at hedgerows crossing  
539 roads (Abbott, 2012), but never at road study sites with different landscape structures and for several  
540 distinctly identified species. In our study, the proportion of flights in the zone at collision risk was rarely  
541 influenced by tree height or distance to trees; nonetheless, increasing distance to trees was associated  
542 with higher proportions of flights at risk for *E. serotinus*, contrary to our expectations. In addition, in  
543 several species (*M. daubentonii*, *P. pipistrellus* and *P. pygmaeus*), taller trees (that generally had their  
544 foliage over the road) led to a higher bat-vehicle co-occurrence.

#### 545 *Traffic*

546 Traffic volume did not affect bat density or position in the zone at risk for most species, contrary to our  
547 expectations. Nonetheless, *P. kuhlii/nathusii* avoided the zone at collision risk more when traffic  
548 increased, possibly because they recognise the danger associated with vehicles, although a specific data  
549 set would be required to test this hypothesis. Moreover, *P. pipistrellus* flew parallel and over the external  
550 sides of the road more often with increasing traffic. These results show that bats spatially avoid the  
551 vicinity of vehicles, completing the observations of Zurcher et al. (2010). The latter did not distinguish  
552 between species, but found that 60% of approaching individuals reversed their course in the presence of  
553 a vehicle.

554 Our results trivially showed that the higher the traffic volume, the higher the temporal bat-vehicle co-  
555 occurrence, probably because bats have no choice but to cross the road closely in time with vehicle  
556 passes when traffic is high. In addition, our study showed that SRE are less likely to fly in the zone at  
557 collision risk when a vehicle is present compared to MRE. It could be that because SRE fly lower and  
558 are more at risk when crossing (Roemer et al., 2017b), they are more reluctant to approach vehicles. In  
559 addition, since the foraging abilities of SRE seem to be more impaired by light and noise than MRE  
560 (Azam et al., 2018; Siemers and Schaub, 2011; Stone et al., 2015), MRE might use roads as foraging  
561 grounds and take more risks than SRE. Therefore, even if SRE are known to fly lower than MRE and  
562 thus at heights more similar to those of vehicles (Berthinussen and Altringham, 2012; Roemer et al.,  
563 2019), their vehicle avoidance behaviour should partially mitigate their susceptibility to collisions. This  
564 result emphasises the importance of accounting for the different aspects of species behaviour when  
565 evaluating their susceptibility to collisions (Chamberlain et al., 2006).

#### 566 *Time of year*

567 Our results show typical activity patterns throughout the year with peak density in summer or autumn,  
568 but it is the first time to our knowledge that it is demonstrated that flight proportion in the zone at risk  
569 at roads increases in autumn (for most species and all guilds). An increased flight proportion in the zone  
570 at risk in autumn could partly be attributed to the naïve behaviour of juveniles, not aware of the danger  
571 of road vehicles. Juveniles are indeed more vulnerable to road collisions than adults (Fensome and

572 Mathews, 2016). This result could also be explained by increasing foraging opportunities on roads  
573 during colder times, as was observed in swallows (Evans et al., 2003). Bats may also be less cautious at  
574 this time of year where they are sexually active (i.e. increases in sexual hormones may decrease  
575 awareness of potential danger (Alcock, 2009)), and where they need to fatten before hibernation.

#### 576 *Species differences*

577 Our study provides detailed information at the species level except for species with small sample sizes,  
578 for which readers are referred to the guild level. Models for guilds also inform on the extent of  
579 generalisation of results because variables selected at the guild level are assumed to exert a significant  
580 influence on several species.

581 Forests clearly stood out as a landscape type with a higher proportion of trajectories in the zone at risk  
582 for MRE, *P. pipistrellus* and *P. kuhlii/nathusii*. For *H. savii* and *E. serotinus*, double parallel tree rows  
583 elicited the smallest proportion of trajectories in the zone at risk. In most species, locations without trees  
584 generated a relatively low proportion of trajectories in the zone at risk.

585 We found a mean number of bat passes at risk of collision per kilometre and per night of 2.3 for SRE,  
586 1024.9 for MRE and 11.7 for LRE. We stress that these figures are necessarily an overestimate since we  
587 could not measure more precisely bat avoidance of vehicles when they were in the zone at collision risk  
588 at less than 10 s from a vehicle pass. In addition, readers have to bear in mind that these figures are not  
589 a proxy for the bat guild susceptibility to road collisions. For this, it is necessary to consider the  
590 proportion of individuals in the zone at collision risk multiplied by the co-occurrence of bats and  
591 vehicles. This calculus placed MRE as the most susceptible bat guild to road collisions. This finding did  
592 not match our expectations since the lowest flyers were always thought to be the most susceptible to  
593 road collisions (Voigt and Kingston, 2016). Fensome and Mathews (2016) found that low-flying species  
594 are more susceptible to collisions, however, it is important to mention that they included both SRE and  
595 MRE in this category. Our results show that MRE are more susceptible than SRE to road collisions  
596 because MRE fly more often in the zone at collision risk and are also more often present in this zone

597 simultaneously to a vehicle pass. This classification, added to species conservation status, can be used  
598 to prioritise conservation actions at roads.

599 *Advantages of conditional probabilities taking into account bat behaviour to assess road collision risks*

600 All quantitative models succeeding the density model were interpreted as conditional probabilities that  
601 an individual is at risk of collision, and their predicted probabilities were multiplied to obtain the overall  
602 bat collision risk if a variable was selected in several of them. The product of all quantitative models  
603 showed that *H. savii* was more at risk of collision at forests and forests edges (and to a lesser extent at  
604 roads without trees), while *P. pipistrellus* was more at risk of collision at perpendicular tree rows. These  
605 products match the patterns of bat density in function of landscape type. The product of quantitative  
606 models also showed that the yearly patterns of collision risks matched the ones of bat density. Collision  
607 risks are more numerous in summer or autumn according to species, and explain the mortality patterns  
608 found in Fensome and Mathews (2016).

609 However, while increasing traffic density was associated with a decrease in SRE density, it was  
610 associated with an increase in the overall collision risk (the product of quantitative models). This  
611 demonstrates, as we expected, that the measure of the number of bat passes can be a good proxy of bat  
612 collision risks in certain contexts, but that it is necessary to also measure bat behaviour to assess collision  
613 risks with certainty in all contexts.

614 In addition, contrary to the classic method of collecting bat carcasses, the results of acoustic flight path  
615 tracking are not biased by predation or observer efficiency, and acoustic flight path tracking may be  
616 applied to study roads after as well as before they are in service, if necessary. It is also well known that  
617 bat carcasses are quite difficult to find (Santos et al., 2011; Slater, 2002) while acoustic flight path  
618 tracking provides a large amount of precise information on bat movements. Yet, out of curiosity, during  
619 field work, we looked for bat carcasses at least once per study site, most often twice (on two different  
620 days), and more rarely up to four times (on four different days). Searches were done along the road on  
621 sections 50 m in length, on each side of the study point. Because it was not the purpose of our study,  
622 searches were randomly done during the day (from 9 am to 9 pm), which has an influence on the finding

623 success since small carcasses are rapidly scavenged (Santos et al., 2011; Slater, 2002). Nevertheless,  
624 only 2 carcasses were found overall (unpublished data). One juvenile female of *Rhinolophus*  
625 *hipposideros* was found on the 12th of August 2016 at study site #11 (dense oak forest on both sides)  
626 and one adult *Pipistrellus pipistrellus* was found on the 7th of June 2016 on study site #55 (“no  
627 vegetation”: some vines and croplands). These results underline the need of using acoustic recordings  
628 to collect enough data per species to attain the aims of our study.

#### 629 *Recommendations for road siting and management*

630 Our first group of recommendations applies to habitat selection during road planning to avoid situations  
631 with enhanced collision risks. As has been recommended in previous studies (Fensome and Mathews,  
632 2016; Medinas et al., 2013), ‘quality habitats’ – depending on species ecology – should generally be  
633 avoided to ensure that roads will avoid habitats with high bat density. However, bat activity is highly  
634 dependent on distance to roost and may be under- or overrepresented at certain habitats according to the  
635 distance to roosts. Therefore, measuring species activity at different seasons on site will always provide  
636 more insights on the potential risks. Moreover, since it is assumed that species do not have comparable  
637 susceptibilities to road collisions (Fensome and Mathews, 2016), possessing information on species  
638 presence and density is highly relevant. The present study also allows us to emit recommendations for  
639 road siting based on the behavioural reactions to landscape features that we measured. Forested areas  
640 should be avoided because they elicit high proportions of flights at risk. Areas without trees should be  
641 prioritised because they almost always led to very low activity levels and low proportions of flights at  
642 risk. However, to explain position at risk, landscape types were only selected in models for species  
643 belonging to MRE and LRE and we cannot conclude on their effect on SRE.

644 Our second recommendation applies to the management of roadside vegetation during construction  
645 work and during the operational phase, to reduce collision risks. A gap of five meters between the road  
646 edge and tree foliage significantly decreased the activity levels of several species across the three  
647 different guilds. Our appreciation of study sites suggests that this effect could be due to higher primary  
648 productivity when vegetation is higher and closer to the road. If less primary biomass is available to

649 insects, foraging opportunities for bats decrease, and so does their density (Threlfall et al., 2012). It is  
650 however controversial to recommend cutting trees at road sides, because this decision will engender  
651 habitat loss in numerous taxa, especially in large-scale impacted areas such as linear transport  
652 infrastructures. Opening habitat at road edges also creates suitable foraging grounds for birds of prey  
653 for instance (Morelli et al., 2014), and will increase their collision probability. It is possible to make  
654 these open verges less attractive by converting them to gravel surface (Kociolek et al., 2015), but this  
655 will eliminate plant habitats. In our results, hard edges also led to higher rates of MRE in the zone at  
656 collision risk. Another possibility for the management of vegetation is thus to only cut a certain number  
657 of trees and clear shrub layers periodically (a frequent practice in French Mediterranean forests to  
658 prevent fires) to reduce primary production and to allow bats to navigate between trees rather than above  
659 the asphalt. The local management will thus depend on the biodiversity stakes of the area. In areas of  
660 high stakes, reducing vehicle speed limit could be an efficient solution, but this was not tested on bats  
661 to our knowledge.

662 Finally, our results allow us to provide insight on a low-cost mitigation measure that has been popularly  
663 proposed to reduce collisions at secondary roads: hop-overs. They consist in planting tall trees at each  
664 side of a road to help bats increase their flight height and cross safely (Limpens et al., 2005). Screens  
665 can be added at each side of the road to prevent bats from crossing at low height. (Christensen et al.,  
666 2016) already found that this measure could be ineffective to help many species crossing roads safely,  
667 as many individuals will just fly around screens to cross. Based on our results, we expect that planting  
668 tall trees next to roads will create new foraging grounds, increase bat density and encourage individuals  
669 to fly in the zone at collision risk if trees are planted very close to the road, as it is often recommended  
670 (Christensen et al., 2016; Voigt and Kingston, 2016). We therefore expect more collision risks with hop-  
671 overs than without, and their use without other measures such as speed reduction should be prohibited  
672 until their efficacy is proven.

673 *Limits of the study and perspectives*



674 Our recommendations can only apply to landscapes and bat communities similar to the ones that we  
675 sampled. Therefore, complementary studies should be conducted in other biogeographical areas (e.g.  
676 Continental or Atlantic areas) to make sure that bats react consistently to the same road landscape  
677 features. However, we expect this endeavour to be quite difficult because of the local particularities in  
678 landscape management. Since we expect bats to be more active at prolific foraging grounds, it would be  
679 interesting to see if the measure of primary production - for example using satellite imaging - can be a  
680 more universal descriptor of bat activity than the description of the local landscape.

681 *Rhinolophus* species are assumed to be very susceptible to road collisions because they fly very close to  
682 ground level (Fensome and Mathews, 2016; Jones and Rayner, 1989; Roemer et al., 2017b). However  
683 *Rhinolophus* species, because of their very high sonar frequencies (Kingston et al., 2000), are very  
684 difficult to detect and to record, and this is why we could not study their flight behaviour with our  
685 method. Acoustic flight path tracking with only two microphones would allow a study of *Rhinolophus*  
686 collision risks, although with simpler metrics (Claireau et al., 2018).

687 Several questions remain unanswered, such as the role of tree species, topography at a medium scale  
688 (i.e. slope of the terrain), and topography at a small scale (i.e. road embankments) in bat collision risks  
689 at roads. The nearby presence of a bat roost is also expected to be an important factor of collisions. At  
690 last, it was reported that juveniles and males are more prone to road collisions (Fensome and Mathews,  
691 2016). It would be interesting to study the behaviour of bats of different age and sex to explain this  
692 finding.

693

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703

#### 704 **Conflict of interest**

705 Biotope is an environmental consultancy involved in road impact assessment studies. Two of the  
706 authors, Charlotte Roemer and Thierry Disca, were employees at Biotope during the time of the study.  
707 They thus declare a financial conflict of interest. Aurélie Coulon and Yves Bas declare that they have  
708 no financial conflict of interest with the content of this article All of the authors take complete  
709 responsibility for the integrity of the data and the accuracy of their analysis. In addition, Aurélie Coulon  
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711

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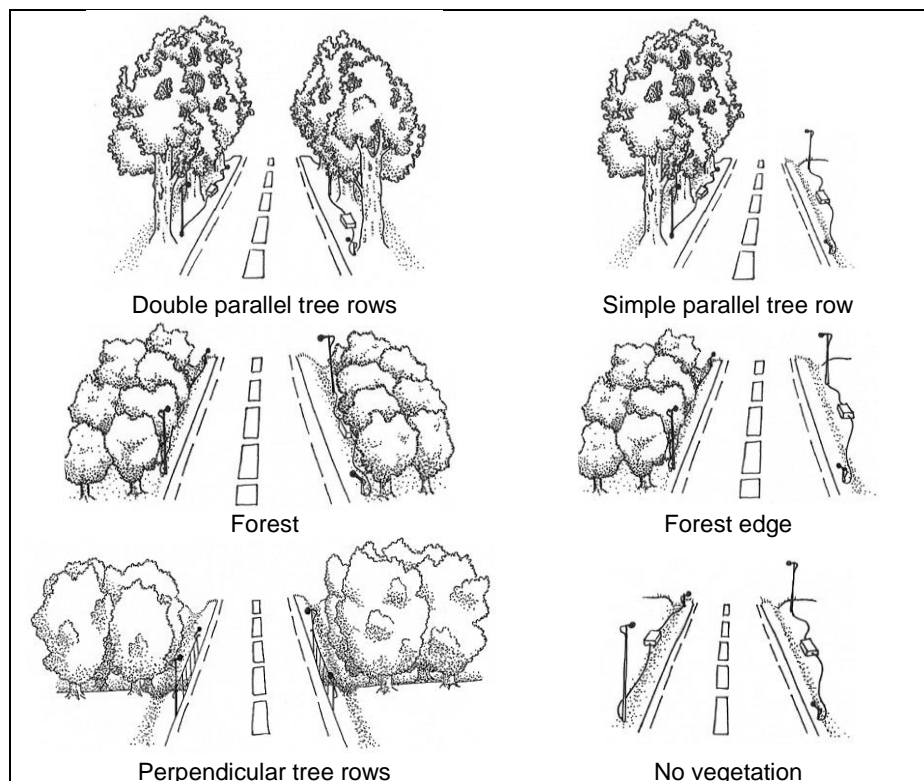
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942 **Supplementary Material**

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945 Figure A 1: Landscape types. Microphones are shown on poles but when possible, they were attached  
 946 to vegetation instead. The box between two microphones is the recorder.

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951 Table A 1: Summary table for the total amount of data in each category and for each species. SRE:  
 952 Short-range echolocators. MRE: Mid-range echolocators. LRE: Long-rang echolocators. N bat passes:

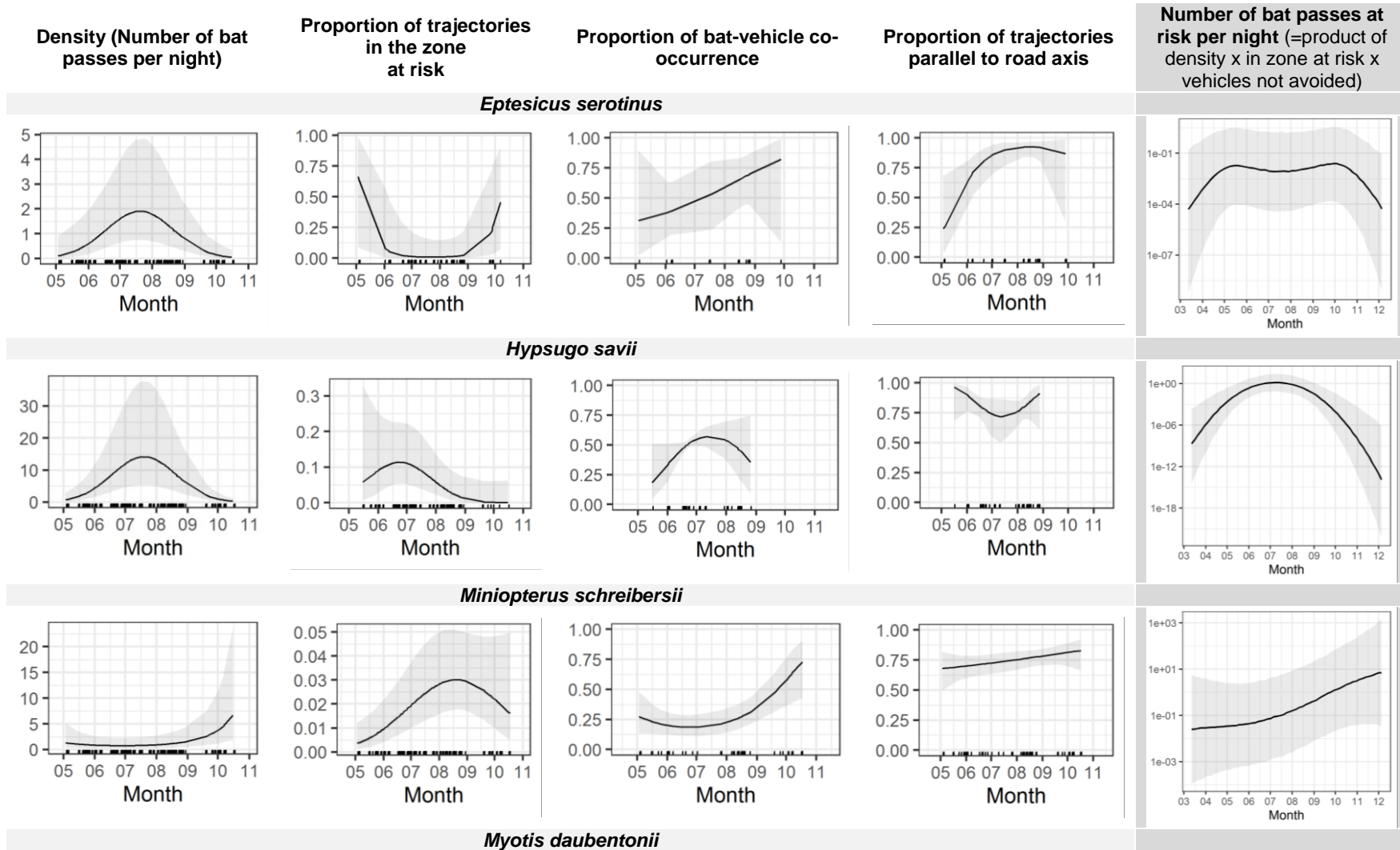
953 rounded median number of bat passes among all four microphones. N flight trajectories: acoustic  
 954 recordings with more than 3 consecutive calls recorded by all 4 microphones. N successful flight  
 955 trajectories: flight trajectories with at least one call in the precision zone (<10 m from microphones  
 956 centroid).

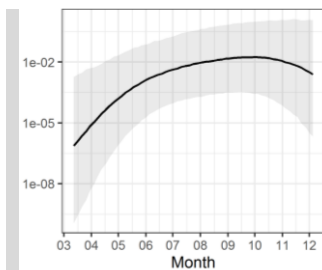
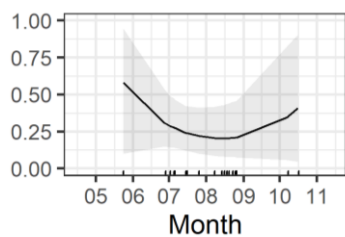
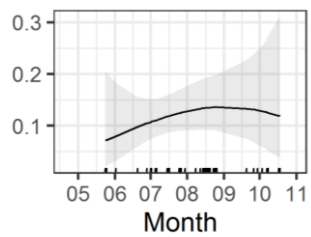
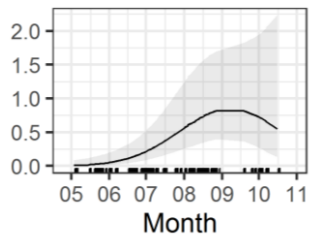
<b>Species</b>	<b>Guild</b>	<b>N bat passes</b>	<b>N flight trajectories</b>	<b>N successful flight trajectories</b>
<i>B. barbastellus</i>	SRE	1157	311	97
<i>M. alcathoe</i>	SRE	2	1	1
<i>M. bechsteinii</i>	SRE	32	11	2
<i>M. capaccinii</i>	SRE	98	40	24
<i>M. daubentonii</i>	SRE	368	287	150
<i>M. emarginatus</i>	SRE	287	29	17
<i>M. blythii/myotis</i>	SRE	1477	583	201
<i>M. nattereri</i>	SRE	345	75	37
<i>Myotis sp.</i>	SRE	405	101	40
<i>Plecotus sp.</i>	SRE	908	281	89
<i>R. euryale</i>	SRE	6	0	0
<i>R. ferrumequinum</i>	SRE	68	2	2
<i>R. hipposideros</i>	SRE	129	3	1
<i>H. savii</i>	MRE	4634	3912	2053
<i>M. schreibersii</i>	MRE	9226	2268	1417
<i>P. kuhlii/nathusii</i>	MRE	45152	28601	11685
<i>P. pipistrellus</i>	MRE	32383	19830	11261
<i>P. pygmaeus</i>	MRE	14410	6136	3033
<i>Eptesicus/ Nyctalus/ Vespertilio</i>	LRE	503	141	31
<i>E. serotinus</i>	LRE	1161	475	107
<i>N. lasiopterus</i>	LRE	13	2	0
<i>N. leisleri</i>	LRE	9306	3506	698
<i>N. noctula</i>	LRE	32	16	5
<i>T. teniotis</i>	LRE	192	53	3
<b>TOTAL</b>		<b>122294</b>	<b>66664</b>	<b>30954</b>



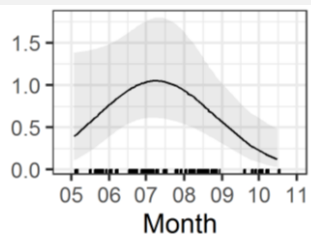
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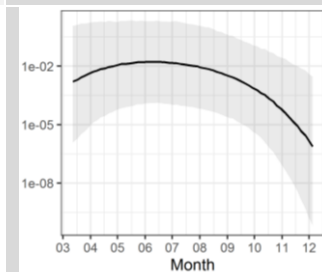
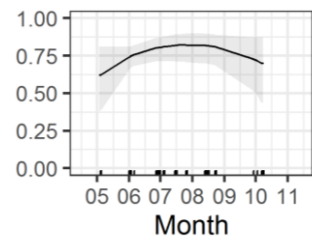
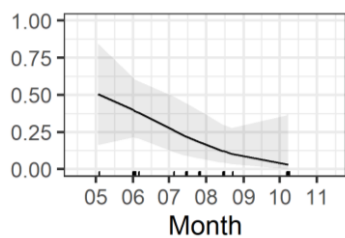
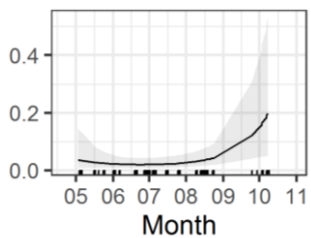
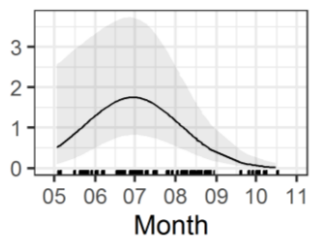




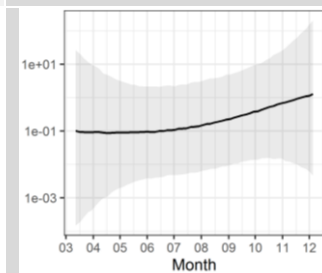
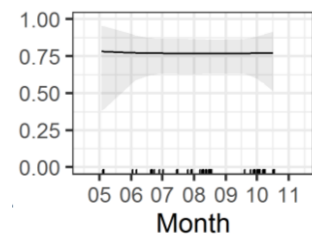
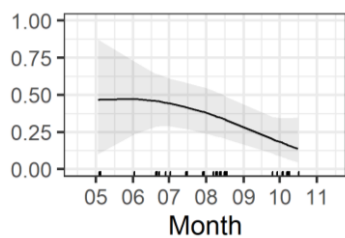
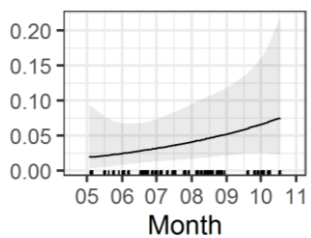
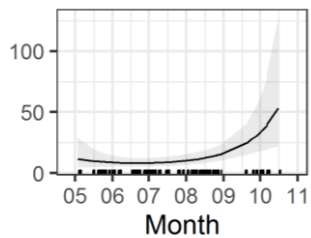
*Myotis emarginatus*



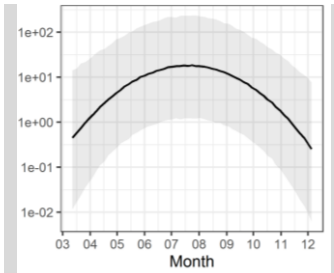
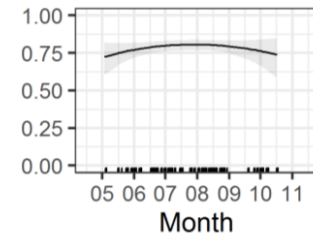
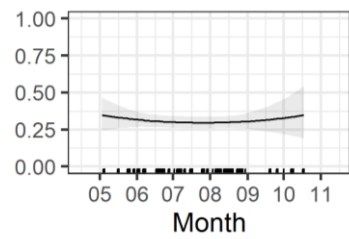
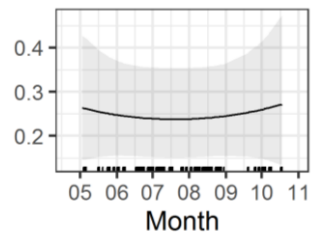
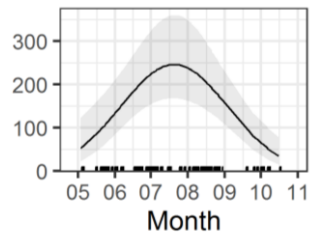
*Myotis myotis/blythii*



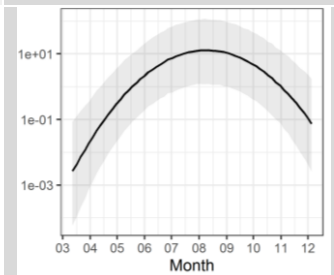
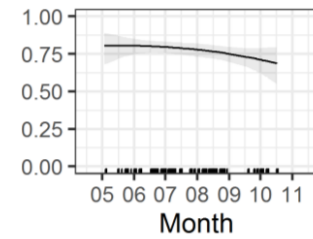
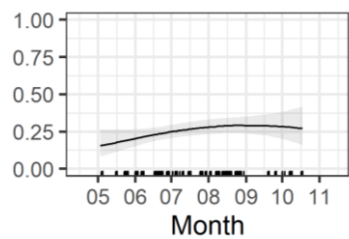
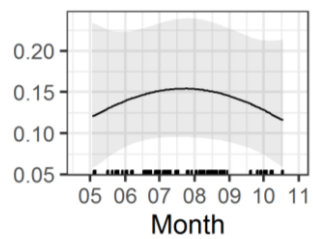
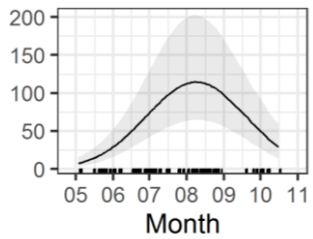
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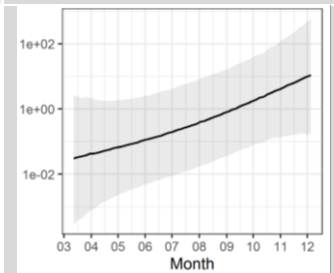
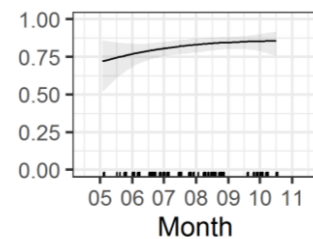
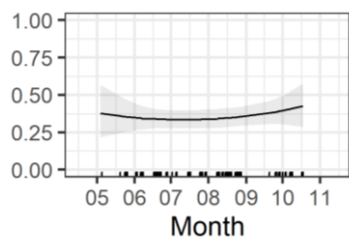
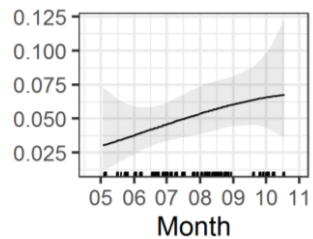
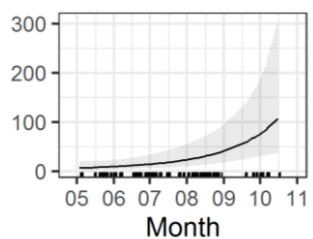
*Pipistrellus kuhlii/nathusii*



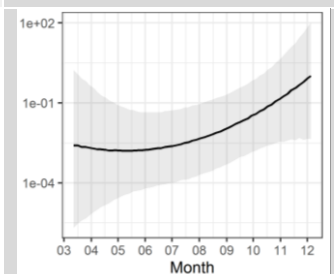
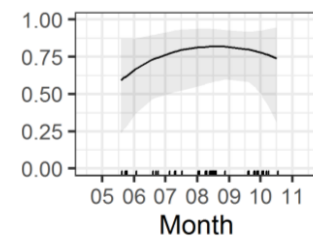
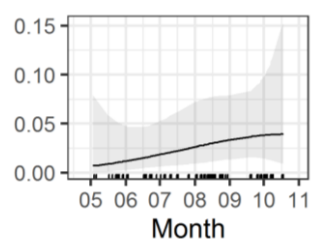
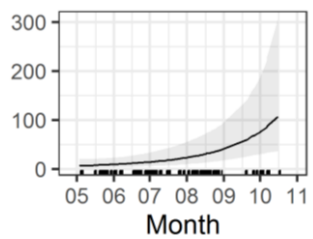
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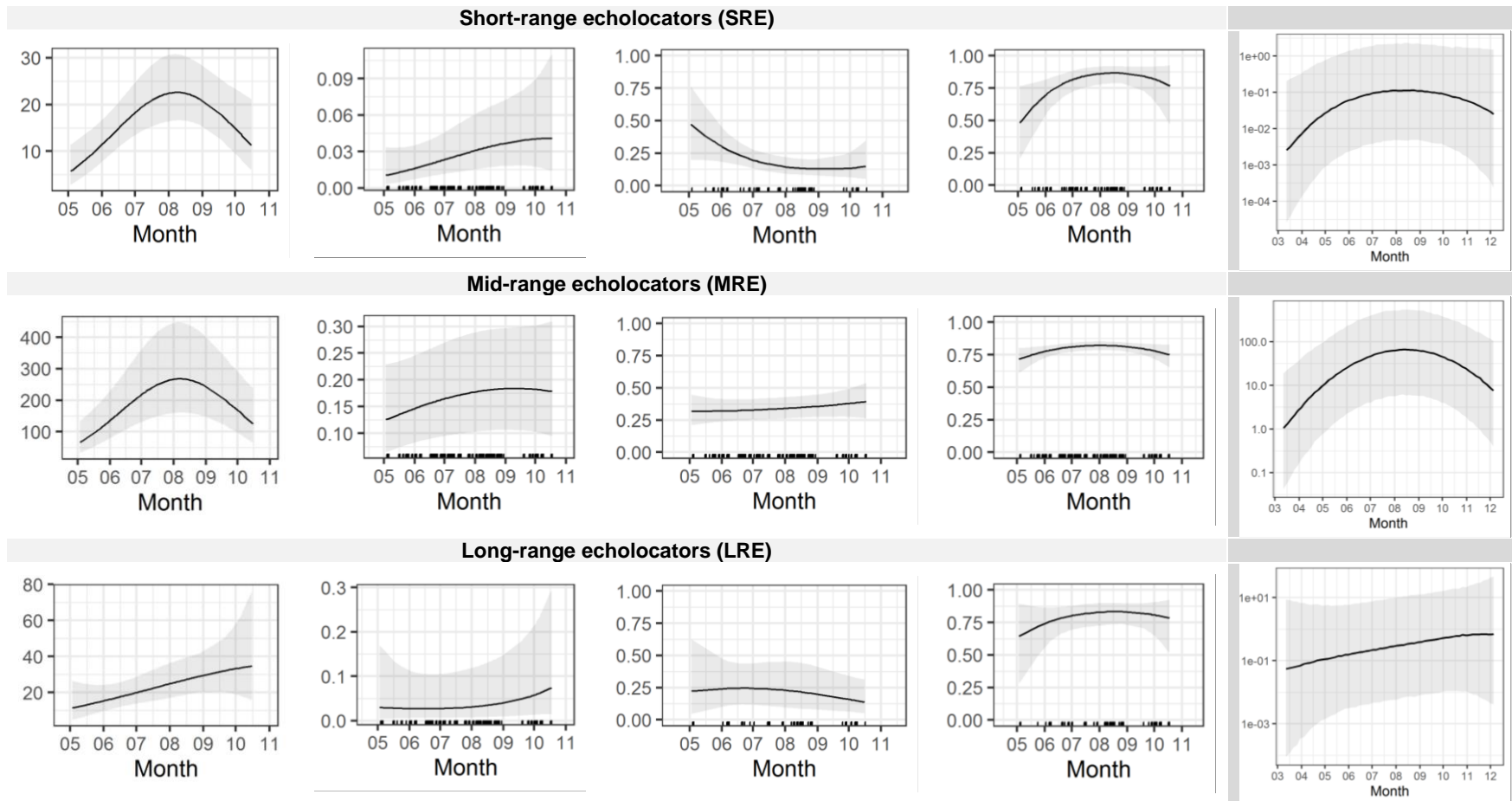


***Pipistrellus pygmaeus***

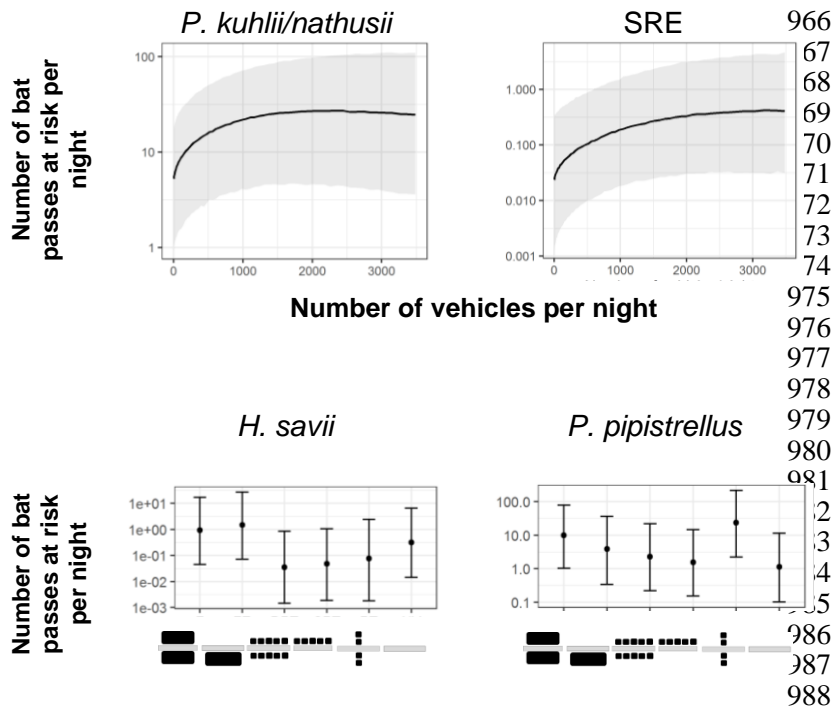


***Plecotus sp.***





960  
 961 Figure A 2: Predicted values for each model and each species or guild in function of time of the year (in month). 95% confidence intervals are shown. The last  
 962 column displays the product of the first three models (density \* in zone at risk \* bat-vehicle co-occurrence) to obtain the number of bat passes at risk per night  
 963 (logarithmic scale). Ticks on the x axis stand for the sampled values.  
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989  
 990 Figure A 3: Product of the first three models (density \* in zone at risk \* bat-vehicle co-occurrence) to obtain the number of bat passes at risk per night (logarithmic  
 991 scale) for variables selected in at least two models for the same species. 95% confidence intervals are shown. Figures represent landscape type viewed from  
 992 the top (road in light grey and trees in black). SRE: short-range echolocators. LRE: long-range echolocators. MRE: mid-range echolocators. F = forest. DPT =  
 993 double parallel tree rows. NV = no vegetation. FE = forest Edge. PT = perpendicular tree rows. SPT = simple parallel tree rows.  
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