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Bat overpasses as an alternative solution to restore habitat connectivity in the context of

road requalification

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Running head: Bat overpasses as a mitigation measure.

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1 Bat overpasses as an alternative solution to restore habitat connectivity in the context of 2 road requalification 3 4 **Abstract** Roads have a multitude of negative effects on wildlife, including their prominent role in 5 habitat fragmentation. Habitat fragmentation particularly affects bats during their nightly 6 movements between roosts and foraging areas. Bat overpasses are among the proposed 7 8 improvements intended to reduce the fragmentation impact of roads, but they have rarely been 9 tested. In this study, we performed a Before-After Control-Impact analysis to assess the 10 efficiency of one bat overpass on the number of bat crossings, by using an acoustic flight path 11 reconstruction (AFPR) approach. We obtained 888 bat crossings of five taxa. Our results showed that the number of bat crossings increased significantly after the installation of the bat 12 13 overpass. Finally, we demonstrated that an overpass correctly placed with respect to a narrow 14 commuting route (a twenty-meter-wide hedgerow) could efficiently restore bat habitat 15 connectivity. 16 Keywords: Acoustic flight path reconstruction (AFPR), bat gantry, before-after control-17 18 impact analysis (BACI), commuting route, crossing structure, mitigation measure. 19 1. Introduction 20 21 Transport has been identified as one of the ten main pressures on biodiversity

(Maxwell et al., 2016) because it contributes to habitat destruction, causes habitat degradation

and fragmentation barrier effects, light and noise disturbance, chemical pollution and direct

mortality by collision with vehicles (Forman and Alexander, 1998; Trombulak and Frissell,

2000). These dramatic changes in landscape configurations have consequences on the overall

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functionality of ecosystems, from individual behaviour all the way up to population dynamics (Krauss et al., 2010; Quinn and Harrison, 1988; Saunders et al., 1991). Indeed, the cumulative ecological effect of the road system on biodiversity at landscape scale (i.e., the road effect-zone) can extend 100 to 800 m depending the type of road, the traffic volume and the habitat crossed by roads (Forman, 2000; Forman and Deblinger, 2000).

Until recently few studies were focused on the impact of roads on bats (see review by Fensome and Mathews, 2016) while, according to their life cycle (i.e. low fecundity, late maturation), population growth rate heavily depends on adult survival. Thus road mortality can impact their local abundance and as a consequence increase their local extinction risk (Medinas et al., 2013). Recent studies have highlighted that roads have a negative impact on the movement and activity of insectivorous bats (Abbott et al., 2015; Berthinussen and Altringham, 2012a; Kitzes and Merenlender, 2014; Zurcher et al., 2010). Indeed, connectivity in the landscape is a key element for bats because it fosters nightly movements between roost and foraging areas (Frey-Ehrenbold et al., 2013). Hale *et al.* (2012) demonstrated that bat activity in a habitat patch (e.g., ponds) increased with the degree of connectivity of the surrounding landscape. Moreover, Pinaud *et al.* (2018) established that bat movements are significantly affected by gap width: the probability of crossing decreases from 80 % for gaps smaller than 10 m to less than 50 % for gaps larger 38 m (i.e. similar to a gap caused by major roads).

In addition to its existing size, the roadway network has experienced a worldwide length increase: 12 million additional lane-km since 2000, and expectations are that there will be further growth of nearly 25 million paved lane-km by 2050 (Dulac, 2013). Hence, there is an urgent need for timely action to facilitate the safe movement of animals across landscapes fragmented by roads or other forms of linear infrastructure, such as the creation of fauna crossing structures to increase landscape connectivity and reduce mortality (Smith et al.,

2015) While there is no legislation that regulate their installation, the number of wildlife crossings have greatly increased in the last 40 years in France: the French motorway network has an average of one fauna crossing structure every 8 km (Carsignol, 2006). Initially intended for wildlife game species, they now respond to a broader demand for biodiversity conservation and particularly protected species such as bats (Council Directive 92/43/EEC of 21 May 1992) which had received relatively little attention.

In order to reduce road impacts and restore habitat connectivity for bats, a range of mitigation measures have been proposed, including bat overpasses (Møller et al., 2016). Bat overpasses are supposed to function as linear features (e.g., hedgerows) that attract and guide bats across the roads above traffic and are thus recommended in the mitigation hierarchy. However, while more bat overpasses are likely to be setup across Europe, Møller *et al.* (2016) highlighted that the current recommendation targets for policy makers and road managers are inadequately implemented or have never had their efficacy been proven. These authors stressed the need for soundly designed research on bat overpasses involving Before-After Control-Impact (BACI) approaches and including behavioural studies to evaluate the efficiency of these overpasses for bats.

Thus, the aim of our study was to evaluate whether bat overpasses, as a recommended mitigation measure, contribute to the restoration of connectivity among habitats severed by roads, using a BACI analysis. We assessed the number of bat crossings where an overpass was installed, using an acoustic flight path reconstruction (AFPR) approach. This overpass bisects a hedgerow in an agricultural landscape in Western France. The experiment was operated from April to September 2016 before the bat overpass was added and from May 2017 to May 2018 after its establishment.

2. Methods

2.1. Study site

The study was carried out in France which experienced a road increase of 12% between 1995 and 2015 (MEEM, 2017), and is expected to see an increase of 0.06% in primary roads by 2030 (i.e., 673 km) (DGITM, 2011).

We studied one bat overpass located in a rural area (intensive farming) near Niort (46°24′W, 0°35′W) on the A83 highway (tarmac; 4 lanes with an emergency lane on each side; speed limit: 130 km/h; annual average daily traffic: 16 218 vehicles in 2015) (Fig. 1). This highway became operational in 2001 and the bat overpass was permanently installed in May 2017.

2.2. Placement and features of bat overpass

The choice of location for the bat overpass followed on from the conclusions of a field study conducted by consulting firm Naturalia Environnement in October 2015. They identified a bat commuting route in an agricultural landscape bisected by a highway. This commuting route is a large hedgerow (a disused railway, closed in 1971, and completely reforested, 20 m wide) that connects two main rivers (Fig. 1).

The bat overpass consists of a traditional gantry for road signs but in this particular case without signs (length: 31,5 m, height: 6 m) (Fig. 1). Instead of road sign, a diamond mesh metal grate was installed on the gantry in order to reflect bat echolocation signals more efficiently (grate height: 1,5 m; diamond mesh: 4,13 X 1,3 cm) (Fig. 1). In addition, ten deciduous trees (height: 4 m; trunk circumference measured at 1 m: 10 to 12 cm) were planted on both sides of the highway in order to guide bats from the hedgerow to the bat overpass.

2.3. Sampling design

For the sampling, we designed a BACI analysis, a sound and effective approach to assess the impact of the installation of the bat overpass. In order to quantify the number of bat crossings, we placed two automatic acoustic recorders: SM2Bat+ (Wildlife Acoustics Inc., Concord, MA, USA), one on each side of the overpass location (i.e., treatment) and in a control site. The control and experimental sites were located as close as possible to each other. As there are no other hedges severed by the highway in the same context in a radius of 5 km, we have positioned the control at 215 m from treatment at the level of the hedge parallel to the road (Fig. 1). Accounting for the species existing in the study area and their respective distance of detection (Barataud, 2015), the distance between the treatment and the control site is sufficient for avoiding the simultaneous recording of the same bat crossing event. This control also takes into account annual and seasonal variations in bat crossings.

Acoustic recorders recorded two independent tracks with 2 microphones spaced 2,1 m to 3,5 m apart plugged into the same recorder (Fig. B.1). One microphone was placed facing the road and the other facing the natural habitat perpendicular to the road. This placement of microphones allowed us to characterize the bat crossings based on the AFPR approach developed in Claireau et al. (2018).

2.4. Acoustic survey

The BACI experiment was conducted from April 2016 to May 2018. We performed the acoustic survey during a period of 25 nights before the installation of the bat overpass (6 sessions from April 2016 to September 2016), and 25 nights following the installation (5 sessions from May 2017 to May 2018). We recorded the treatment and control site simultaneously for 4 or 5 successive nights each month (from 30 min before sunset to 30 min after sunrise). The detectors were set to automatically trigger in response to any sound at a

frequency of between 8 and 192 kHz and a signal-to-noise-ratio (SNR) level above 6 dB (more information in appendix A).

2.5. Species identification

Recordings were processed and automatically analysed with *Tadarida* (Bas et al., 2017). This software toolbox automatically detects and extracts sound features of recorded call sequences and classifies them according to a confidence index if emitted by a defined species or group of species using Random Forest as a classification algorithm.

In addition to the calls assigned to *Pipistrellus pipistrellus* and *Barbastella barbastellus*, we defined three groups: *P. kuhlii/nathusii*, *'Eptesicus/Nyctalus'* (*Eptesicus serotinus* and *Nyctalus* spp.) and 'gleaners' (*Plecotus* spp. and *Myotis* spp.) as contacts with these taxa were difficult to identify with certainty at the species level (Obrist et al., 2004). Moreover, suspect bat calls involved in bat crossings detected by the AFPR approach were checked manually using BatSound© software (Pettersson Elektronik AB, Sweden).

2.6. Statistical analyses

In our study, we quantified the number of bat crossings at the treatment and at control site before and after the installation of the bat overpass. The response variable in our analyses was 'Bat crossings'. The explanatory variables were the 'Pair' (i.e., control or treatment) and the interaction between 'Pair' and 'Period' (i.e., before or after). According to the nature of our response variable (count data and potential over-dispersion), we performed a generalized linear mixed model (GLMM) with the *glmer* function (R package *lme4*) with a negative binomial error distribution and a log link (Zuur et al., 2009). We included a random effect on 'Date' allowing us to implicitly account for the conditions of nights, such as the effects of

weather, the temporal correlation and the seasonal effects. The full model was written asfollows:

'Bat crossings' ~ 'Pair' + 'Pair:Period' + 1|'Date'

We evaluated the quality of our model by comparing it to the null model using Akaike's information criterion (AIC) (Mac Nally et al., 2017).

3. Results

Among the 888 bat crossings detected: 75,8 % were assigned to *P. pipistrellus*, 11,5 % to *P. kuhlii/nathusii*, 7,3 % to *B. barbastellus*, 3,8 % to 'Eptesicus/Nyctalus' and 1,6 % to gleaners (Table B.1 & B.2). Bat activity measures at treatment and control sites were typical for this agricultural landscape (see appendix C).

We detected more bat crossings at the treatment than at the control (P < 0.001, Table 1), and we did not find a change of bat crossings between the two periods (i.e., before and after the bat overpass establishment) at the control site (P = 0.165, Table 1) while we detected an increase of bat crossings between the two periods at the treatment site (P = 0.001, Table 1). These significant interaction effects indicated a positive effect as a result of the bat overpass on bat crossings for the whole set of bats analysed together ($\chi^2 = 15.264$, df = 2, P < 0.001) (Table 1). For the following species or group: P. pipistrellus, P. kuhlii/nathusii and B. barbastellus, results showed a similar effect (Table 1).

Regarding taxa for which the establishment of the overpass had a significant positive effect on bat crossings, there was a difference of at least 24.5 points in AIC between the model and the null model (Table 1).

4. Discussion

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To our knowledge, our experiment is the only one based on a BACI design concerning bat overpasses as mitigation measure. This robust and powerful approach demonstrated that bat crossings increased significantly after the installation of the bat overpass and thus, that the overpass likely contributed to the restoration of habitat connectivity.

In the case of operational highways, the installation of a bat overpass on a bat commuting route bisected by the road, two studies found this mitigation measure is an insufficient solution (Berthinussen and Altringham, 2012b; Claireau et al., 2018) whereas in our case, we found that this mitigation measure can increase bat habitat connectivity. One major difference that could explain differences in results between our study and previous ones is the sampling design. Previous studies did not provide a measure of bat crossings before the installation of bat overpasses and thus, did not per se investigate if these structures can restore or not the habitat connectivity. A non-exclusive alternative hypothesis is the importance of the placement of bat overpasses (i.e. in our study the bat overpass was placed with respect to a narrow commuting route). The importance of the position of the overpass in relation to commuting routes has already been highlighted (Claireau et al., 2018), but it still remains a challenge to develop a methodology for identifying the best location to construct overpasses. Although methods focusing on landscape connectivity appear as a very promising approach (Mimet et al., 2016), with the current knowledge, we advocate for the construction a bat overpass on the bat commuting route identified during the Environmental Impact studies, underlying the crucial importance of the accuracy of these EIAs.

However, there is an urgent need to confirm our results through replications of such design with different overpass structures and landscapes. Furthermore, even if we have shown that bat overpasses can aid in the restoration of habitat connectivity in our landscape context (i.e. agricultural landscape dominated by intensive farming), we would still need to assess

whether or not the bats fly at a safe height to know if the bat overpasses are actually efficient. To answer this issue, it will be necessary to specifically study bats flight height, using flight path reconstruction and the same framework (i.e. a before/after study).

5. Conclusions

Pending confirmation of similar findings in further studies that bat overpasses restore commuting routes, overpasses constitute a possible mitigation for restoring habitat connectivity where hedgerows are bisected by large roads. Nevertheless, we highly recommend replicating this BACI study design in other study sites to confirm our findings. Finally, for future road-construction, we advocate to investigate a Before-During-After Control-Impact (BDACI) design such as recommended in Roedenbeck et al. (2007). Ideally, the efficiency of the restoration of connectivity should be evaluated against the level of connectivity prior to the construction of the road.

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215 **Data accessibility** The acoustic data are posted with the citizen science programme "VigieChiro" 216 (https://vigiechiro.herokuapp.com/) 217 218 **Appendices** 219 220 The appendix A the additional material and methods; the appendix B, the additional results; the appendix C, the known bat activity in the study area. They may be found in the 221 222 online version of this article. 223 224 References Abbott, I.M., Berthinussen, A., Stone, E., Boonman, M., Melber, M., Altringham, J., 2015. 225 226 Bats and roads, in: Handbook of road ecology. John Wiley & Sons, Ltd., pp. 290–299. 227 Barataud, M., 2015. Acoustic ecology of european bats; species identification, Study of their habitats and foraging behaviour, Inventaires & biodiversité. National Museum of 228 Natural History, Paris; Biotope, Mèze. 229 230 Bas, Y., Bas, D., Julien, J.-F., 2017. Tadarida: a toolbox for animal detection on acoustic recordings. J. Open Res. Softw. 5, 8. https://doi.org/10.5334/jors.154 231 Berthinussen, A., Altringham, J., 2012a. The effect of a major road on bat activity and 232 diversity: Effect of a major road on bat activity. J. Appl. Ecol. 49, 82–89. 233 https://doi.org/10.1111/j.1365-2664.2011.02068.x 234 235 Berthinussen, A., Altringham, J., 2012b. Do bat gantries and underpasses help bats cross 236 roads safely? PloS One 7, e38775. 237 Carsignol, J., 2006. Routes et passages à faune; 40 ans d'évolution (No. 0641w), Les 238 rapports. SETRA. 239 Claireau, F., Bas, Y., Puechmaille, S.J., Julien, J.-F., Allegrini, B., Kerbiriou, C., 2018. Bat 240 overpasses: An insufficient solution to restore habitat connectivity across roads. J. Appl. Ecol. https://doi.org/10.1111/1365-2664.13288 241 DGITM, 2011. Schéma national des infrastructures de transport. Ministère de l'Écologie, du 242 243 Développement durable, des Transports et du Logement. Dulac, J., 2013. Global land transport infrastructure requirements; Estimating road and 244 railway infrastructure capacity and costs to 2050. International Energy Agency. 245 246 Fensome, A.G., Mathews, F., 2016. Roads and bats: a meta-analysis and review of the 247 evidence on vehicle collisions and barrier effects. Mammal Rev. 46, 311–323. 248 https://doi.org/10.1111/mam.12072 249 Forman, R.T.T., 2000. Estimate of the area affected ecologically by the road system in the United States. Conserv. Biol. 14, 31–35. https://doi.org/10.1046/j.1523-250 251 1739.2000.99299.x

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313 **Tables**

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Table 1: Estimates, standard errors and *P* of the number of bat crossings at the control and the treatment before and after the bat overpass

establishment. The control and the after period are the intercept in this model's summary.

	All bats			P. pipistrellus			P. kuhlii/nathusii			B. barbastellus			'Eptesicus/Nyctalus'			Gleaners			
	β	SE	p-value	β	SE	p-value	β	SE	p-value	β	SE	p-value	β	SE	p-value	β	SE	p-value	
(Intercept)	-0.438	0.310	0.158	-1.886	0.440	<0.001	-2.888	0.665	<0.001	-20.826	23.869	0.383	-1.127	0.485	0.020	-19.481	3075.657	0.995	
PairTreatment	2.994	0.182	< 0.001	3.958	0.324	< 0.001	3.106	0.594	<0.001	20.938	23.871	0.380	-0.975	0.454	0.032	17.886	3075.657	0.995	
PairControl:Periodbefore	-0.665	0.479	0.165	-0.466	0.699	0.505	0.962	0.805	0.232	17.005	23.890	0.477	-2.844	1.124	0.011	15.812	3075.658	0.996	
PairTreatment:Periodbefore	-1.313	0.378	0.001	-1.237	0.442	0.005	-1.094	0.496	0.027	-1.371	0.489	0.005	-0.085	0.726	0.907	-0.465	0.726	0.522	
AIC full model		535.4			428.3			236.5			163.0			143.5			83.8		
AIC nul model		569.0			1211.6	I		261.0			204.5			143.2			90.1		

Anova(GLMM)

	All bats			P. pipistrellus			P. kuhlii/nathusii			B. barbastellus			'Eptesicus/Nyctalus'			Gleaners			
	Chisq	df	p-value	Chisq	df	p-value	Chisq	df	p-value	Chisq	df	p-value	Chisq	df	p-value	Chisq	df	F	p-value
Pair	346.920		1 < 2.2e-16	196.574	1	< 2.2e-16	29.097	1	0.000	8.775	1	0.003	3.429	1	0.064	2.321		1	0.128
Pair:Period	15.264		2 <0.001	9.382	2	0.009	11.112	2	0.004	8.430	2	0.015	6.699	2	0.035	0.409		2	0.815

Figure with legends

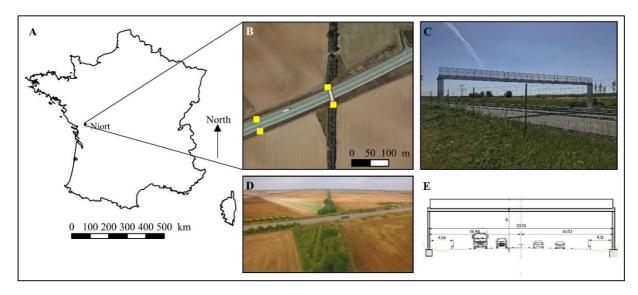


Figure 1: A. Location of study site. B. Sampling plan: acoustic recorders (yellow squares)
and bat overpass (solid blue line). C. Picture of the gantry D. Picture of the study site before.

E. Technical drawing of the gantry (Source: Institut national de l'information géographique et forestière, Koox-production/Vinci-autoroutes).