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Bat overpasses: an insufficient solution to restore habitat connectivity across roads

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Abstract

1. Roads have many negative effects on wildlife, including their role in habitat fragmentation. Habitat fragmentation affects bats during their daily movements between roosts and foraging areas. As bats are protected in Europe, developers must implement specific mitigation measures that are hierarchically structured to achieve a null net impact. However, very few specific mitigation measures have been undertaken specifically for bats. Bat overpasses (e.g. gantries) are among proposed improvements intended to reduce the impact of roads, but they have rarely been tested. The effectiveness of overpasses in facilitating safe road crossing of bats is critical for justifying the implementation of this mitigation measure. We therefore assessed whether bat overpasses are effectively used by bats.

2. We studied three bat overpasses with different designs in France. We developed an innovative method to characterize bat crossings using acoustic flight path reconstruction (AFPR). We used six pairs of stereo acoustic recorders in different habitat types that were located on both sides of the road, and operated simultaneously throughout the night.

3. Recording data contained 57 941 bat passes and 284 bat crossings from six species of bats at the three study sites. Our results suggest that crossings are more numerous if an overpass is located where bat commuting routes have been identified by environmental impact assessment. However, we found that the proportion of bat crossings along the commuting route was the same with or without an overpass; thus highlighting that bat overpasses do not fully restore habitat connectivity.

4. *Synthesis and applications.* Our study demonstrates that acoustic flight path reconstruction (AFPR) is a useful approach to obtain information on bat flight behaviour. We also emphasize the importance of field testing the effectiveness of mitigation measures, such as those intended to offset the impact of roads on biodiversity, and highlight that such measures should not be implemented based on their theoretical effectiveness alone.

Résumé (FR)

1. Les routes ont de nombreux effets négatifs sur la faune, y compris leur rôle dans la fragmentation des habitats. La fragmentation des habitats affecte les chauves-souris au cours de leurs déplacements journaliers entre leur gîte et leurs zones d'alimentation. Les chauves-souris étant protégées en Europe, les aménageurs doivent mettre en œuvre des mesures de réduction des impacts afin d'obtenir un impact net nul. Cependant, peu de ces mesures sont dédiées aux chauves-souris. Les chiroptéroducs figurent dans ces mesures et visent à réduire l'impact des routes, mais ils ont rarement été testés. Mesurer leur efficacité pour faciliter la traversée des chauves-souris en toute sécurité au-dessus de la route est essentielle pour justifier leur mise en œuvre. Nous avons donc évalué si les chiroptéroducs sont utilisés par les chauves-souris.

2. Nous avons étudié trois chiroptéroducs au design différents en France et avons développé une méthode innovante pour caractériser les franchissements de chauves-souris en les localisant grâce à leurs cris d'écholocation. Nous avons utilisé six paires d'enregistreurs stéréo acoustiques dans différents types d'habitat situés des deux côtés de la route enregistrant simultanément toute la nuit.

3. Les enregistrements ont permit d'obtenir 57 941 séquences de chauves-souris et 284 traversées de chauves-souris pour six espèces sur les trois sites d'étude. Nos résultats suggèrent que les franchissements de chauves-souris sont plus nombreux si un chiroptéroduc est situé là où ont été identifiés les corridors lors de l'évaluation environnementale. Cependant, nous avons constaté que la proportion de franchissement de chauves-souris le long du corridor était la même avec ou sans chiroptéroduc; soulignant ainsi que les chiroptéroducs ne restaurent pas complètement la connectivité des habitats.

4. *Synthèse et applications.* Notre étude démontre que la reconstruction des trajectoires de vol de chauves-souris en acoustique est une bonne approche pour obtenir des informations sur le comportement de vol des chauves-souris. Nous soulignons également l'importance de tester sur le terrain l'efficacité des mesures de réduction, telles que celles visant à compenser l'impact des routes sur la biodiversité, et soulignons que de telles mesures ne devraient pas être mises en œuvre uniquement sur la base de leur efficacité théorique.

Keywords

Acoustic flight path reconstruction, bats, Chiroptera, collision, crossing structures, habitat connectivity, habitat fragmentation, mitigation measures.

Introduction

Transport has been identified as one of the ten main pressures on biodiversity (Maxwell *et al.* 2016) because it contributes to habitat destruction, degradation and fragmentation, barrier effects, light and noise disturbances, chemical pollutions and direct mortality by collision with vehicles (Forman & Alexander 1998; Trombulak & Frissell 2000). These dramatic changes in landscape configuration have many consequences, ranging from individual behaviours and population dynamics to the overall functioning of ecosystems (Saunders, Hobbs & Margules 1991; Krauss *et al.* 2010).

At the national scale, the quantification of road network density suggests that populations of some animals, such as large snakes, may be decline by 50% or more due to road-associated mortality (Rudolph *et al.* 1999). For insectivorous bats, road mortality can increase the risk of local extinction because these animals have low fecundity, late maturation and a population that depends on adult survival (Medinas, Marques & Mira 2013). In addition to road mortality, high night-time traffic can actually lead to the reduced use of breeding habitats near the motorway by acting as a barrier to forest habitats, and this road-effect zone operates well beyond 1000 m from the road (Eigenbrod, Hecnar & Fahrig 2009). Thus, roads and traffic can reduce the persistence of wildlife populations, particularly when they restrict the access of individuals to breeding sites or foraging habitats (Forman *et al.* 2003).

Since 2000, the worldwide roadway network length has increased by approximately 12 million lane-km, and globally roads are predicted to grow by nearly 25 million paved lane-km by 2050 (Dulac 2013). Hence, there is an urgent need to facilitate the safe movement of animals across landscapes fragmented by roads or other forms of linear infrastructure. One method used to reduce road-associated mortality is the creation of fauna crossing structures (Smith, van der Ree & Rosell 2015).

Most studies on road effects have focused on terrestrial mammals and amphibians, and until recently, few studies have focused on the effects of roads on flying animals, such as birds and bats. However, recent studies have highlighted that roads have a negative impact on the activity and movement of insectivorous bats (Zurcher, Sparks & Bennett 2010; Berthinussen & Altringham 2012b; Bennett & Zurcher 2013; Kitzes & Merenlender 2014; Abbott *et al.* 2015; Fensome & Mathews 2016). Indeed, connectivity in the landscape that allows daily movements between roosting and foraging areas is a key element for bats (Frey-Ehrenbold *et al.* 2013; Pinaud *et al.* 2018) For example 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.

In the European Union, all bat species are strictly protected by the Habitats Directive (Council of the European Union 1992), the most influential nature conservation framework in Europe (Fontaine *et al.* 2007). Developers must demonstrate that they will establish mitigation measures to prevent, reduce, and compensate for impacts that result in any loss of bat foraging or roosting habitats (Bezombes *et al.* 2017). The mitigation measures that have been proposed to reduce road impacts are overpasses (e.g. bat overpasses), underpasses (e.g. viaducts), vehicle speed reduction, deterrence and diversion (e.g. planting hedges), artificial roosting sites (e.g. bat boxes) and habitat improvement (Møller *et al.* 2016). Recent studies have suggested that wildlife crossings and underpasses could be the best solutions to restore ecological continuity, whereas bat overpasses seem to be less effective (Berthinussen & Altringham 2012a; Abbott, Butler & Harrison 2012; Møller *et al.* 2016).

Bat overpasses are presumed to function as linear features (e.g. a hedgerow) that will attract and guide bats across the roads above traffic and have been recommended by environmental impact assessments (EIA). However, Møller *et al.* (2016) highlighted that carefully designed research on bat overpasses and controlled testing, including studies of the behaviour of individual species, were needed to scientifically evaluate the effectiveness of these overpass designs for bats.

Studies on the effectiveness of mitigation infrastructure for bats are particularly scarce; only one peer-reviewed study has been performed to evaluate the effectiveness of bat gantries (a form of overpass) (Berthinussen & Altringham 2012a), and few technical reports were identified in Møller *et al.* (2016). These studies found that the current recommendations for policy makers and road managers are inadequately implemented or have never been proven to be effective. These uncertainties emphasize the critical need to further test the efficacy of these mitigation methods, which are likely to be implemented across Europe and potentially further afield.

Our overall aim was to evaluate whether bat overpasses, as a recommended mitigation measure, contribute to the restoration of connectivity between habitats fragmented by roads. To achieve this aim, we developed a new methodology based on acoustic flight path reconstruction (AFPR) to better characterize bat flight trajectories in the vicinity of three bat overpasses and to evaluate their effectiveness in restoring habitat connectivity. More specifically, we tested two different overpass designs among three sites to determine whether (i) bats cross the road at the location of bat overpasses, (ii) bat crossings at bat overpasses were more numerous than at other nearby unmitigated commuting routes identified in the EIAs, (iii) bat overpasses placed on known commuting routes were used more frequently than bat overpasses that were located outside of the route and (iv) bat overpass designs influence the effectiveness of bat crossings when the overpasses are placed along known commuting routes.

Materials and methods

Study site

The study was undertaken in France, which experienced a 12% increase in the length of roads between 1995 and 2015 [from 962 000 km to 1 078 000 km (MEEM 2017)], with an additional 673 km planned to be in place by 2030 (DGITM 2011).

We studied three bat overpasses located in rural areas mainly surrounded by woodlands and grasslands. Two overpasses were in the Rhône-Alpes region near Lyon, named Millonnais (ML) (45°50'N, 4°12'E) and Moulin-Paris (MP) (45°51'N, 4°14'E), both crossing the A89 highway which became operational in 2013. These two sites were separated by 2.3 km (Fig. 1) and bat overpasses have been permanently installed on this highway since November 2012. The third overpass was in the Picardie region near Beauvais, named Troissereux (TR) (49°28'N, 2°3'E) on the road D901 (Fig. 1). Construction of the TR road, which occurred between 2015 and 2016, led to the bisection of a large natural wildlife corridor. The mitigation measures planned in the EIA consisted of (i) building a wildlife crossing and (ii) installing a bat overpass at the location where the road bisected the ecological corridor during road construction. Both infrastructures were built in parallel with our monitoring (Fig. S1.1 S1.2) before the road became operational in December 2016. The characteristics of the roads are detailed in Table S1.1.

Known bat communities and bat commuting routes

During the aforementioned EIAs, bibliographic searches and acoustic surveys (passive and/or active) were conducted for each site by consulting firms (methods detailed in appendix 1). The main objectives were to detect protected species and identify biodiversity issues (locations of commuting routes, roosting bats and foraging areas) in the areas that would be impacted by the road work.

The bat community at ML and MP included the following species: *Pipistrellus pipistrellus*, *P. kuhlii*, *Eptesicus serotinus*, *Plecotus spp.* and *Myotis myotis/blythii.* A hibernacula of *Barbastella barbastellus*, which is a species of conservation concern, was also reported 700 m away from the A89 (approximately 330 individuals) (Letscher, Prat & Vincent 2007). At TR, the bat community was composed of *P. kuhlii*, *P. nathusii*, *P. pygmaeus*, *M. nattereri*, *M. mystacinus*, *M. daubentonii*, *M. bechsteinii*, *M. myotis*, *Myotis sp.*, *Nyctalus noctula*, *N. leisleri*, and *Plecotus spp.* Furthermore, in 2012, an important breeding colony of approximately 370 individuals of *M. myotis* was located in a castle of Troissereux, 1.2 km away from the study site (Dupuy & Yobokre 2014).

Given the methodologies implemented in the aforementioned EIAs (appendix 1), i.e. the absence of quantitative measures of bat activity along the commuting route and the absence of a control site, it was not possible to re-use the data collected before construction to perform a before/after or a control/impact analysis.

Placement and features of bat overpasses

To mitigate the fragmenting effect of the road and restore connectivity, two overpasses (MP & TR) were placed within the bat commuting routes identified during the preconstruction EIA. Due to technical constraints (the soil had insufficient bearing capacity), the third overpass (ML) was built 325 m away from the identified commuting route (Fig. 1). This preconstruction commuting route was situated at the lowest part of a valley (with a stream) which was backfilled for the road to cross the valley. The stream now flows through a culvert with a 1-metre diameter that was obstructed by vegetation during our surveys, precluding bats from using it. Thus, bats had to cross above the road (Fig. S1.3 S1.4).

The designs of the bat overpasses were different but were not specifically tested due to the confounding effect of road traffic. Indeed, while the TR overpass had a unique design - wires with polystyrene balls - and was studied at a site without traffic, the two other sites had similar designs but were studied on a highway that was in operation. ML and MP were designed as U-shaped metal structures (Fig. 2 A, B, C). At MP, according to the experts who wrote the EIAs, a grid parallel to the road was installed to help bats to better detect the overpass and to force them to fly at a safe height across the road (Fig. 2 D). The last overpass (TR) comprised two tensioned wires vertically spaced 1.2 m apart with polystyrene spheres each spaced at short intervals on the wires (Fig. 3). These polystyrene spheres were designed with a reflective micro-surface to optimize the likelihood of bats receiving sonar echoes while flying (Fig. 3 B). The wires were stretched between two tall trees and connected to the forest edge. The characteristics of the three bat overpasses are detailed in the Table S1.2.

Sampling design

To assess the use of bat overpasses by bats at each site, we placed two automatic acoustic recorders per site (Song Meter SM2Bat+, Wildlife Acoustics Inc., Concord, MA, USA), one on each side of the overpass (henceforth referred to as a 'pair'). Acoustic recorders were used to capture stereo recordings with microphones (SMX-US and SMX-U1, Wildlife Acoustics Inc., Concord, MA, USA) spaced 3.5 m apart and connected to the same recorder. The microphone on the left channel was always placed facing the road, whereas the microphone on the right channel was placed perpendicular to the road, facing natural habitat (e.g. agricultural land, hedgerow) (Fig. 4 A). This placement permitted us to characterize the bat crossings based on the AFPR approach.

To determine if bat crossings were more numerous at overpasses compared to other crossings that occurred in the vicinity, we also placed pairs of acoustic recorders spaced at least 25 m apart ($\bar{x}$: 77.6 m; min: 25.2 m; max: 129.7 m). These additional pairs were placed at each site in the main habitat types present in the vicinity of the overpass (Fig. 1). One pair was placed at a location with agricultural land on both sides of the road. This pair was our control pair because open agricultural lands are typically poorly utilised by the bat community considered (Kerbiriou *et al.* 2018).

At the site level, this sampling involved the use of six pairs of acoustics recorders placed in the following six habitats: (i) overpass (O), (ii) forest on both sides of the road (F/F) (iii) stream on both sides of the road (S/S), (iv) forest edge on one side and agricultural land on the other side (F/A), (v) forest on one side and the hedgerow on the other side (F/H), and (vi) agricultural land on each side of the road (A/A).

Acoustic survey

Ultrasonic recordings were collected in the spring and summer of 2016 for four successive nights for TR and five successive nights for ML and MP (at the end of May for TR, in July for ML, and in September for MP) under favourable weather conditions, i.e. without rain, with low wind speed (< 7 $m.s^{-1}$) and at temperatures higher than 12°C, as recommended by the French national bat-monitoring programme.

The acoustic surveys were performed for the whole night from 30 min before civil sunset to 30 min after civil sunrise. At the site level, all twelve acoustic recorders were operating simultaneously. The detectors were set to automatically record in real time all sounds with frequencies greater than eight KHz. We used a trigger level threshold of 6 dB signal-to-noise ratio for frequencies between 8 and 192 KHz.

As we used two models of omnidirectional ultrasonic microphones, we performed tests to compare their sensitivities. The results showed no significant difference between microphones (bat pass duration and triggering distance); hence, the data have been analysed without considering the model of microphone as a covariate.

Species identification

To identify the species from acoustic recordings, we first used Kaleidoscope© software (Wildlife Acoustics Inc., Concord, MA, USA) to extract .wav files from the recorded .wac files. A time expansion factor of 10 was specified, and we split channels using five seconds as a maximum duration.

Then, we analysed the ultrasound recordings with the software *Tadarida* (Bas, Bas & Julien 2017), which identifies species-specific echolocation calls. This software automatically detects and extracts sound feature parameters of the recorded echolocation calls and classifies them into known classes according to a probability value that a call is from a specific group/bat species using a random forest algorithm (Cutler *et al.* 2007).

All bat calls involved in bat crossings (bat crossings were detected by the AFPR approach, see below for more details) were checked manually using BatSound© software (Pettersson Elektronik AB, Sweden). In addition to the calls assigned to *P. pipistrellus*, *E. serotinus* and *B. barbastellus*, we constructed three groups (*P. kuhlii/nathusii*, *Plecotus spp.* and *Myotis spp.)*, as contacts with these taxa were difficult to identify with certainty (Obrist, Boesch & Flückiger 2004).

Detection of bat crossings using acoustic flight path reconstruction (AFPR)

To determine the number of bat crossings, we developed an innovative method using AFPR [two open source scripts in R (see appendix 2) and online repository: https://github.com/FabienClaireau].

For each acoustic recorder, we calculated the time difference of arrival (TDOA) for each bat pass, allowing the recorder to detect whether a bat crossed the median plane of the two microphones and in which direction it went. To calculate the TDOA, we needed to obtain the time-frequency features of each echolocation call. For that purpose, the .wav files were processed by the open software *Tadarida-L* 1.0.2 (Bas, Bas & Julien 2017; github.com/YvesBas/Tadarida-L).

When the bat was in the left plane, closer to the road than the median plane, the TDOA was a positive value and vice versa (Fig. 4 B). When a change of sign was observed in more than 10% of the calls, we assumed that the bat had crossed the median plane. According to the spatial design of our microphones (i.e. at a right angle to the road), we could determine whether a bat from outside the road was headed towards the road (TDOA changing from negative to positive), or if a bat coming from the road was exiting the road (TDOA changing from positive to negative) (Fig. 4 C). Hence, to identify a bat crossing, the detection of a bat on all four microphones was required.

Then, we used species identity and the time elapsed as the two criteria to match the entering and exiting flights on each side of the road to detect road crossing events. The time elapsed ($\bar{x}$: 23.21 s; min: 13.1 s; max: 38.6 s) was defined according to the distance between the paired acoustic recorders and the expected bat flight speed (approximately 4-15 $m.s^{-1}$) (Fig. 4 C). A pair of acoustic recorders can detect bats at an average distance of 25 m for common species, such as *Pipistrellus spp.* (Barataud 2015). This detection distance must be taken into account for the placement of pairs of acoustic recorders along the road to avoid recording and hence counting the same bat with two separate pairs.

Statistical analyses

Our sampling design generated the number of bat crossings in different habitat types around the road and bat overpass. In the case where bats randomly crossed the road in any habitat type, we did not expect to detect any significant variations in bat crossings between these habitats. However, in the case where a bat overpass was used more frequently by bats to cross the road than other areas, we expected a greater number of bat crossings at the location of the bat overpass than within the surrounding habitat. Finally, if bats continued to cross the road by moving along the commuting route rather than using the overpass (Fig. 1), we expected to have a greater number of bat crossings along the commuting route.

The first analysis assessed whether there was an overall effect of bat overpasses on bats (placement and features were combined such as the design of bat overpass, traffic and type of road). For this purpose, we evaluated the habitat types that contained the greatest number of bat crossings, and compared the number of crossings among them. This analysis was performed for all pairs in the three sites with a generalized linear mixed model [GLMM, R package glmmADMB (Skaug *et al.* 2014)]. The response variable in our analyses was the number of bat crossings for all species (bat crossings) and the explanatory variable was the habitat type (n=6). According to the nature of our response variable (count data), we performed a zero-inflated GLMM with a negative binomial error distribution (link=log) (Zuur *et al.* 2009). We included a first random effect of date in the model because all recorders ran simultaneously on the same night, allowing us to implicitly account for the conditions that night, such as the effects of weather. In addition, we included a second random effect of the pair of acoustic recorders nested in the site to account for the hierarchical sampling design. Bat crossings at the overpass were used as the reference (i.e. intercept) in the model. The difference in the number of survey nights between sites was taken into account with the random effect structure (pairs nested in site). The full model was written as follows:

Bat crossings ~ Habitat types + 1|Date + 1|Site/Pairs

As we only had one explanatory variable, there was no model selection process. However, we compared it to a null model based on the Akaike weight of each model. We aimed to evaluate whether the quality of our model was good by comparing it to the null model (including only the random effects) using Akaike's information criterion (AIC) (Mac Nally *et al.* 2017).

The second analysis assessed the effect of each single bat overpass by determining whether the number of bat crossings was higher at each overpass than in the surrounding habitat. We applied a similar GLMM (i.e. zero-inflated, negative binomial and a random effect on date), but performed the analysis separately at each site. Thus, we used one full model for each site. The response variable in our analyses was the number of bat crossings for all species (bat crossings) and the explanatory variable was composed of the pairs of acoustic recorders (pairs). Bat crossings at the overpass were used as the reference (i.e. intercept) in each model. The full model was written as follows:

Bat crossings ~ Pairs + 1|Date

We also compared this model to a null model based on the AIC. Finally, when we wanted to compare the sites, we used the ratio between bat crossings and bat passes in each site. Thus, the difference in the number of recording nights between sites did not bias the results.

Results

Bat detections and bat crossings

For the three study sites, we recorded 57 941 bat passes (for details at the site level, see Table S3.1); within these bat passes, we detected 284 bat crossings (ML: 37, MP: 67 and TR: 180) for the six evaluated species. Species of conservation concern identified by EIAs [*B. barbastellus* (for ML and MP) and *M. myotis* (for TR)] were detected during our survey. However, detections were rare, precluding meaningful statistical analysis at the species level. Among the 284 bat crossings detected, the species with sufficient occurrences for the assessment of bat road crossings were *P. pipistrellus* at 73% (n=208) and *P. kuhlii/nathusii* at 22% (n=61). Details on bat crossings are presented in Table S4.1 and S4.2.

At ML, 37 bat crossings were detected; 19% occurred at the bat overpass (n=7), whereas 65% occurred where the road crossed the commuting route identified by the EIA (n=24). The remaining 16% of the bat crossings were located in other habitat types (n=6). At MP, 67 bat crossings were recorded; 39% were detected where the overpass was installed (n=26), whereas 51% occurred where the road crossed the previously identified commuting route (n=34). The remaining 10% of the bat crossings were located in other habitat types (*n*=7). At TR, 180 bat crossings were recorded; 54% were detected where the overpass was installed (*n*=97), whereas 43% occurred where the road crossed the previously identified commuting route (*n*=77). The remaining 3% of the bat crossings were located on agricultural land (control sample) (*n*=6).

Effect of the overpasses on flight crossings

Our analyses demonstrated that bats do not cross the road randomly. Our control pair of microphones, i.e. agricultural land on both sides of the road ('A/A'), recorded the lowest number of bat crossings (Fig 5, Table S4.1). Furthermore, when we analysed the overall effect of bat overpasses in comparison to the surrounding habitat without overpasses, we found no difference in the number of bats crossing between the bat overpass and the stream on both sides of the road, or between the bat overpass and the forest/hedgerow (Table 1), which were two categories with limited samples (Table 1). As the habitat type with the stream on both sides of the road ('S/S') is only present in one site, with only one pair of microphones replicated for five nights, we ran the model without these data and found similar results (Table S5.1). Moreover, it is noteworthy that we found similar patterns when we used the ratio of crossings (i.e. the ratio between the number of bat crossings and bat passes) as the response variable instead of the number of bat crossings (Table S5.2).

Then, at the site level (for MP and TR), bat crossings were more numerous at the location of the bat overpass in comparison to the other locations (Fig 5, Table S4.1). At MP, we found significantly fewer bat crossings at the control pair of microphones ('A/A') ($P<0.001$), at forest/agricultural land ('F/A') ($P<0.001$), and for two of three pairs on both sides of the road in the same forest ('F/F') ($P<0.01$; $P<0.001$) compared to the bat overpass (Fig. 5 B1). At TR, we found significantly more bat crossings at the bat overpass than at all the other pairs ($P<0.001$) (Fig. 5 C1). However, at ML, we did not detect any difference in the number of bat crossings between the bat overpass and other habitat types (Fig. 5 A1).

It is also noteworthy that we had a change in AIC greater than 10 between the full models and the null models (Table S5.3).

Bat crossings at the overpass and at the unmitigated commuting route

When we compared the number of bat crossings at the bat overpass to the sum of the crossings at other locations without overpasses along the commuting corridor (as identified during the EIA), we obtained conflicting results. Considering the length of the bat commuting route and our sampling design (number of pairs within commuting route and interval distance between pairs), we covered approximately 57% of the width of the commuting route at ML, 39% at MP and 100% at TR.

At ML, where two pairs were placed along the commuting route and where the overpass was built 325 m away from the commuting route identified by the EIA, bats crossed the road 3.4 times more often at the unmitigated commuting route than at the location of the bat overpass (Fig. 5 A1). At MP, where four pairs were placed along the commuting route (including the bat overpass), bats crossed the road 1.3 times more frequently at the sites along the commuting route that did not include the overpass (forest) than at the location of the bat overpass (Fig. 5 B1). At TR, where four pairs were placed along the commuting route (including the bat overpass), bats crossed the road 1.3 times more frequently at the location of the bat overpass than at the rest of the commuting route without the overpass (forest) (Fig. 5 C1). Finally, we found similar patterns for the most abundant species, *P. pipistrellus* and *P. kuhlii/nathusii* (Fig. S6.1).

Again, the full models had an AIC 10 points higher than the null model (Table S5.3).

Discussion

Bat crossings at the bat overpasses

We demonstrate that some bats cross the road at the locations of bat overpasses. However, this does not prove their effectiveness at restoring habitat connectivity because these numbers could not be compared to the pre-bat overpass construction numbers.

Bat crossings at the bat overpasses compared to the surrounding habitat including the unmitigated commuting route

Overall, our results show that bat overpasses are more successful when they are placed in a location with forested areas on both side of the road and that they are avoided when placed in open habitats such as farmland. The avoidance of flight across open areas is common in numerous species (Frey-Ehrenbold *et al.* 2013). In contrast, we found no difference between the number of bat crossings at the location of the overpass and at pairs with a stream on both sides of the road ('S/S'). This was expected as it is well documented that bats use linear aquatic habitats to commute and forage (Hale *et al.* 2012). At ML, bat crossings were 3.4 times more frequent along the commuting route identified by the EIA study than at location of the bat overpass. Surprisingly, we recorded twice as many bat crossings at microphone pair E with forest-agricultural land (n=16) than at pair F, where the stream is present on both sides of the road (n=8) (Fig. 5 A1, Fig. S1.3 S1.4, Table S4.1). These results suggest a modification of the main commuting route after the construction of the road (pair F to pair E). This interpretation cannot be confirmed in the absence of a Before-

After-Control-Impact (BACI) approach, emphasizing the need to include the AFPR methodology at the earliest stage of EIA and not only post-construction.

At MP and TR, 57% and 55%, respectively, of the bat crossings were at the location of the bat overpass, which was installed along the commuting route identified during the EIAs. Even though we detected two to six times more crossings at the location of the bat overpass compared to any other pair individually (Fig. 5 B1 C1, Table S4.1), when the number of bat crossings across the commuting route (excluding those along the overpass) were summed, we detected the same proportion of bat crossings compared to the overpass (MP: 43%; TR: 44%). Furthermore, bat crossings along the commuting route are probably underestimated because the pairs did not cover the full width of the commuting route at ML and MP (57% and 39% coverage). Thus, the number of bat crossings along the unmitigated part of the commuting route is higher than presently estimated for these two sites.

Finally, although we do not know whether the proportion of bat crossings at the commuting route after the construction of the road was the same as it was after the installation of the overpasses, we believe that this mitigation measure failed to fully restore habitat connectivity, since a large proportion of bat crossings occurred along the commuting routes and not at the overpass. Overall, bat crossings are dispersed over the entire width of the commuting route (100 to 330 m in length, Fig. 1, Fig. 5) and are not restricted to overpass sites.

Influence of bat overpass placement and/or their features

The ML bat overpass was built 325 m away from the identified commuting route because of technical constraints. Our results demonstrate that bats did not make a detour to cross the road at the location of the bat overpass. Bats continued to cross the road where the commuting route was identified before the construction (Letscher 2007). Hence, although this is based on a limited number of sites investigated, a bat overpass is more likely to fail to restore connectivity when it is not positioned where the commuting route was identified.

In the case of bat overpasses placed within a commuting route (MP & TR), our results showed a greater use of the overpass at TR than at MP. Indeed, the ratio between bat crossings and bat passes at MP was 15 times lower than that at TR. The first hypothesis to explain these results is the absence of vehicular traffic at TR. A second non-mutually exclusive hypothesis is the influence of the overpass design, which seems to be more effective when it consists of ropes with polystyrene balls stretched between tall trees than when it is a U-shaped metal structure. The absence of a gap between the bat overpass and the trees possibly allows a better continuity with the forest edge. Moreover, the wires at TR were positioned directly at the flight height of the bats (<2 m). A third non-mutually exclusive hypothesis is the influence of the road profile; at TR, the shoulders are higher than the road, while at MP, one side is lower than the road while the other is higher. These results suggest that the effectiveness of overpasses placed within the commuting route is likely to be influenced by several variables and their interaction (e.g. topography, habitat), highlighting the need for further research.

Limitations and benefits of the AFPR approach to identifying bat crossings

To detect bat crossings, we developed an innovative method using an AFPR approach. However, this method did not allow us to measure whether bats fly at safe heights above traffic when crossing at bat overpasses. Furthermore, not all bat crossings are detected. Indeed, the condition that must be met to obtain a bat crossing is that the bat is detected by the four microphones. This is highly linked with the distance of detection of the bat (Barataud 2015), which varies among species according to the average intensity of their calls (in dB) and the directionality of the sound emitted. This constraint is important for species that have a very narrow echolocation system oriented forward such as *Rhinolophus ferrumequinum* [distance of detection of 5 m (Barataud 2015)]. Regardless of the habitat type considered, the proportion of bat crossings detected were very low compared to the number of bat passes recorded (2% of the number of bat passes were bat crossings). A field trial would be necessary to objectively compare the automated AFPR approach with direct field observations based on thermal imaging videos.

Although the AFPR method only detected a subset of the bat crossings, there is no reason to believe the pattern observed (i.e. variation between pairs or habitat types or with vs without an overpass) would be biased towards greater/lower detection in different habitats or environments.

Usability of the AFPR approach

AFPR is suitable for a repeatable non-biased BACI studies design (e.g. before and after traffic is introduced to a new road) and it does not depend on the experience of the experimenter, in contrast to direct visual observations, which require extensive training. This innovative method could be included in EIA studies to perform before and after surveys. Such a flight behaviour monitoring system can also be included in EIAs concerned with any kind of development projects, including, for example, wind turbine establishment (Roemer *et al.* 2017). Most consulting firms already have such equipment (acoustic recorders for stereo recordings) for bat species inventories and can use the methodology developed herein. The use of this method allows automated monitoring throughout the night and does not require the attendance of specialized staff, which drastically reduces the cost of the study. Moreover, the software *Tadarida* and our scripts are open source and freely available.

Management implications

Some bats crossed the road at the location of bat overpasses that were installed within their commuting routes. However, our results demonstrate that the two types of bat overpasses investigated in this study did not fully restore habitat connectivity, despite being specifically designed to do so.

These conclusions also highlight the importance of the EIA to propose measures to effectively avoid fragmentation (e.g. changing the road layout). However, if avoidance is impossible, wildlife overpasses or underpasses (especially the latter) should be favoured (Møller *et al.* 2016). It is imperative to carry out monitoring prior to road construction to identify commuting routes used by bats.

Moreover, as shown here at one site, road construction can modify bat commuting routes. As bats seem to avoid making a detour to use bat overpasses to cross the road (O'Connor & Green 2011; Berthinussen & Altringham 2012a; Czerniak *et al.* 2013; Schut *et al.* 2013), it seems imperative to correctly place the overpass where the post-construction commuting route will be. To determine the placement of these mitigation measures, EIAs should use a BACI design, i.e. identifying the existing flight path and quantifying the flow of bats along a commuting route before and after road construction (Møller *et al.* 2016). The before phase provides an initial state against which it is possible to evaluate the efficacy of the mitigation measures, and post-construction monitoring should be compulsory.

Due to a lack of knowledge, the implementation of mitigation measures without clear evidence for their effectiveness is, unfortunately, not uncommon, highlighting the need already expressed in the literature to monitor offset measures over time (Quétier, Regnery & Levrel 2014). To fill in this knowledge gap and provide environmental impact assessment studies with scientific data on which to base their recommendation, before and after analyses should be systematically carried out and the data made publicly available to inform meta-analyses.

Authors' contributions

FC, YB, SJP, BA and CK devised the study. FC, YB and CK implemented the study. FC collected the data. FC and YB wrote the scripts to characterize the bat crossings. FC and CK analysed the data and led the writing of the manuscript. All authors critically contributed to the drafts of the manuscript and gave their final approval for its publication.

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Data accessibility

Acoustic data are available via the Dryad Digital Repository https://doi.org/10.5061/dryad.vn36290 (Claireau *et al.* 2018).

Raw acoustic data are also archived and available via the French citizen science programme “VigieChiro”(http://vigienature.mnhn.fr/page/participer-vigie-chiro), at the portal http://vigiechiro.herokuapp.com/ with the following site ID: 420395, 420420, 420421 and 600548.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix 1: Additional information about the study sites

Appendix 2: Scripts used for the AFPR

Appendix 3: Detailed information on bat activities for each species, site and points

Appendix 4: Bat crossings

Appendix 5: Bat crossings adjusted for bat activity and comparison of full models to a null model

Appendix 6: Bat crossings for *P. pipistrellus* and *P. kuhlii/nathusii*

Table

Table 1. Estimates, standard errors (SE) and *P* for the number of bat crossings according to habitat types for each pair of detectors at each site. Comparisons of bat crossings between overpass pairs and the other pairs were calculated at the site scale by GLMM (Bat crossings ~ Habitat types + 1|Date + 1|Site/Pairs). Bat crossings at the overpass were used as the reference (i.e. intercept) in the model. The estimates and SE were the gaps at the intercept. Back-transformed values were the values corresponding to the category.

Habitat types per pair	*n*	Estimates	SE	z value	*P*	Back-transformed value
Overpass (intercept)	14	1.812	0.481	/	/	6.123 (3.785, 9.905)
Agricultural land / Agricultural land	18	-2.954	0.547	-5.4	<0.001	0.319 (0.172, 0.593)
Forest / Agricultural land	15	-1.026	0.508	-2.02	0.04358	2.197 (1.240, 3.892)
Forest / Forest	27	-0.869	0.38	-2.29	0.02216	2.570 (1.602, 4.125)
Forest / Hedgerow	5	-1.362	0.832	-1.64	0.10165	1.568 (0.658, 3.740)
Stream / Stream	5	-0.833	0.77	-1.08	0.27916	2.664 (1.191, 5.960)

Figures' legend

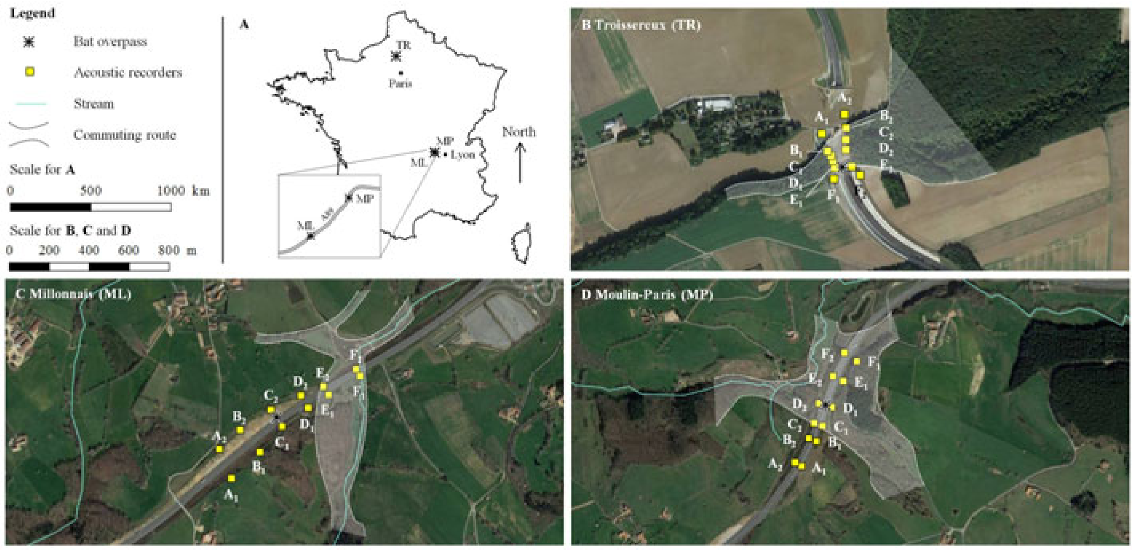
Figure 1. A. Location of the three study sites with an enlarged image of each site with the locations of the overpasses (**B**, **C** and **D**) and the locations and numbers of acoustic recorders in different habitat types and along the commuting route for bats. Image source: Google Maps (October 2017).

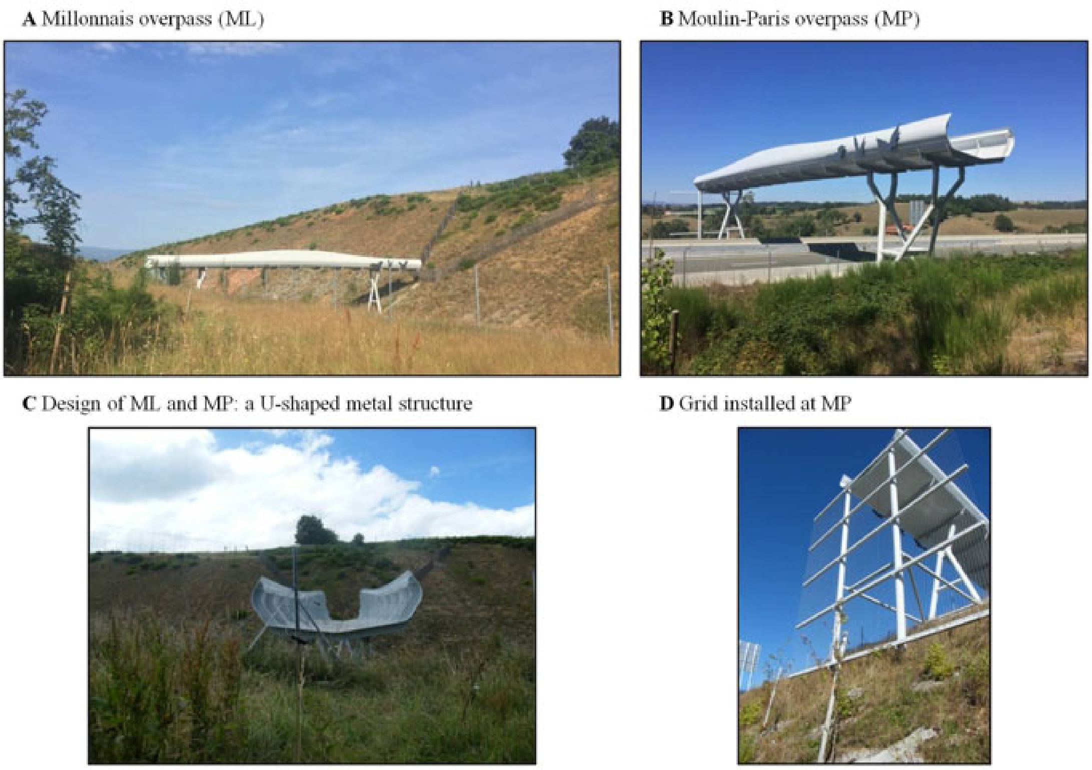
Figure 2. Pictures of bat overpasses permanently installed on highway A89 (ML and MP) near Lyon.

Figure 3. Pictures of bat overpass temporarily installed during road construction on road D901 (TR) to the north of Paris.

Figure 4. A. Positions of the microphones: the left channel (mic 1) facing the road and the right channel (mic 2) facing the habitat context and perpendicular to the road. **B.** Calculation of the time difference of arrival (TDOA). **C.** We defined a crossing as when a bat that entered the road on one side was detected exiting the road on the other side. As it was not possible to identify individual bats based on their commuting/foraging calls, we matched entering and exiting using species identity and time elapsed.

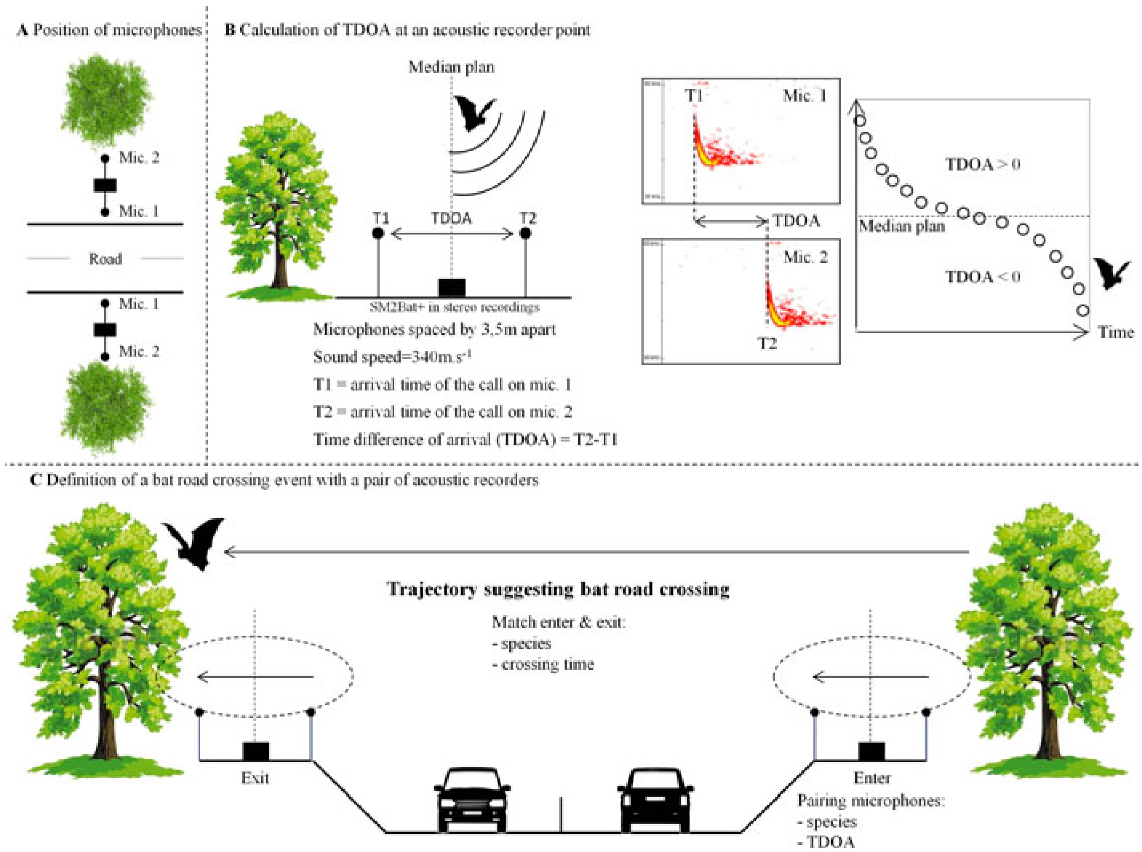
Figure 5. Number of bat road crossings per night for all bats (raw data) per pair of acoustic recorders per overpass (A, ML; B, MP; C, TR). Table S4.1 lists the number of bat crossings per pair. The habitat types (A, agricultural land; F, forest; H, hedgerow; S, stream; CR, commuting route) are included under the number of pairs. Comparisons of bat crossings between overpass pairs and the other pairs were conducted at the site scale by GLMM (Bat crossings ~ Pairs +1|Date). Bat crossings at the overpass were used as the reference (i.e. intercept) in each model (***, $P<0.001$; **, $P<0.01$; *, $P<0.05$).


Legend
Bat overpass
Acoustic recorders
Stream
Commuting route
Scale for A
0 500 1000 km
Scale for B, C and D
0 200 400 600 800 m
A
TR
Paris
MP
ML
Lyon
A89
North
B Troissereux (TR)
C Millonnais (ML)
D Moulin-Paris (MP)

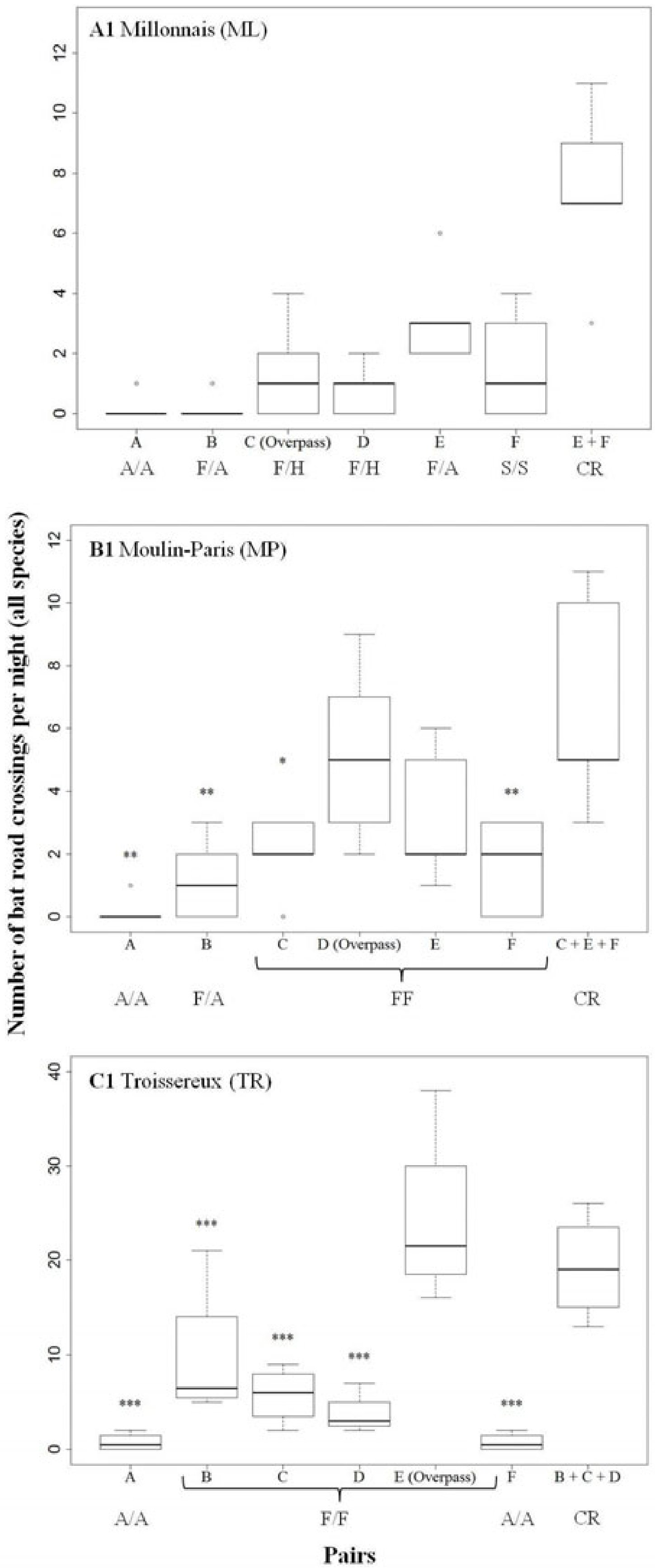

A Millonnais overpass (ML)
B Moulin-Paris overpass (MP)
C Design of ML and MP: a U-shaped metal structure
D Grid installed at MP

A Troissereux overpass (TR)

B Design of TR: two tensioned wires with polystyrene spheres



A Position of microphones
Mic. 2
Mic. 1
Road
Mic. 1
Mic. 2
B Calculation of TDOA at an acoustic recorder point
Median plan
T1
TDOA
T2
SM2Bat+ in stereo recordings
Microphones spaced by 3,5m apart
Sound speed=340m.s-1
T1 = arrival time of the call on mic. 1
T2 = arrival time of the call on mic. 2
Time difference of arrival (TDOA) = T2-T1
T1
Mic. 1
TDOA
Mic. 2
T2
TDOA > 0
Median plan
TDOA < 0
Time
C Definition of a bat road crossing event with a pair of acoustic recorders
Trajectory suggesting bat road crossing
Match enter & exit:
- species
- crossing time
Exit
Enter
Pairing microphones:
- species
- TDOA


A1 Millonnais (ML)
A
B
C (Overpass)
D
E
F
E + F
A/A
F/A
F/H
F/H
F/A
S/S
CR
B1 Moulin-Paris (MP)
A
B
C
D (Overpass)
E
F
C + E + F
A/A
F/A
FF
CR
C1 Troissereux (TR)
A
B
C
D
E (Overpass)
F
B + C + D
A/A
F/F
A/A
CR
Number of bat road crossings per night (all species)
Pairs