We thank the recommender for the time invested in this review, and for the very helpful comments and suggestions. Please find our answers in the present document.

Round #2

by Gloriana Chaverri, 2020-10-30 20:15
Manuscript: https://www.biorxiv.org/content/10.1101/2020.07.15.204115v1 version 1

Minor revisions for the preprint titled "Influence of local landscape and time of year on bat-road collision risks"

Dear Charlotte and co-authors,

I thank you for making the changes suggested by the reviewers on your preprint titled "Influence of local landscape and time of year on bat-road collision risks". I believe you have adequately addressed all of our suggestions; however, new ones have arisen from your changes and from a more thorough revision of the entire text. For this new version I decided not to send it out for review again, but would like you to carefully consider my following suggestions before I give the preprint my recommendation.

Line 20: remove moreover. Line 23: Remove "bats avoiding vehicles or not" from the text, and keep it to what you can measure (bat-car co-occurrence). Line 42: change maximal to greatest.

➔ We edited the manuscript according to the recommender's suggestion.

Lines 42-43: this sentence is confusing, as it seems that there might only be these two options. Do you mean that bats followed the road axis regardless of the type of habitat present?

➔ The sentence is indeed confusing. We changed it to "Finally, bats mainly followed the road axis regardless of the type of landscape."

Line 57: change enhances to increases (remove increasing at the beginning of this line). Line 58: put comma after parenthesis.

➔ We edited the manuscript according to the recommender's suggestion.

Lines 58-59: What do you mean by "results concerning the role of traffic or speed limit on road-kills were CONTRASTED for other vertebrate taxa"?

➔ Indeed, the sentence is here again confusing. We changed it to "They showed for example that road width, traffic and/or speed limit increases collisions in large mammals (Nelli et al., 2018; Neumann et al., 2012; Seiler, 2005; Valero et al., 2015), but traffic and speed limit either increased or decreased road-kills in other vertebrate taxa (Clevenger et al., 2003; D'Amico et al., 2015; Mazerolle, 2004)."

Lines 60-62: Please modify this sentence. My suggestion is: Studies on a variety of animal groups also found that preferred habitats for foraging or movement, described at the home-range scale (e.g. presence or absence of woodland, cropland, wetland …), are more often…

➔ We edited the manuscript according to the recommender's suggestion.

Line 64: I think here you are referring to habitat rather than landscape features.

➔ We edited the manuscript according to the recommender's suggestion.

Line 76: Do you mean here that gaps in vegetation are an important factor that is known to increase road collisions?

➔ Indeed, that is what we mean, thus we edited the sentence for more clarity: "For example in birds, gaps in vegetation are an important factor known to increase road collisions (Lin, 2016; Orłowski, 2008)."

Line 93: Change qualified to classified. Line 101: Add road here, for example "decreases bat road crossing".

➔ We edited the manuscript according to the recommender's suggestion.

Lines 107-109: You need a reference to support this claim. Doing this here is very important since you are not providing any evidence in the introduction, or elsewhere, that flight direction is relevant for understanding collision risk.

➔ We suggest making clearer that parallel flight involves longer flight path in the zone at risk than perpendicular flight. Since it is a geometric evidence, we feel it does not need any reference: "But they did not take into account bats flying parallel to the road axis, although this behaviour may be a determinant factor of collisions, because ~~in this situation bats spend much more time in the zone at collision risk.~~ bats flying parallel to the road axis may fly at risk of collisions for dozens of meters, while crossing a road only implies flying at risk of collision for a few meters."

Lines 121-125: This last part does not seem to be adding anything to the main topic of this paragraph, so I'd suggest you remove it.

➔ We agree that this part could be removed. However, it was added to fit the request of Mark Brigham, who asked to calculate an estimate of the number of potential collisions. This calculus helped us classify bat guilds in function of their susceptibility to road collisions, and we now think that this new result is in fact one of the highlights of our study.

➔ We propose to simplify this part as following: "Per capita mortality is also a very useful tool to prioritise conservation actions in function of the susceptibility of species to anthropogenic impacts. For instance, it was shown that bats of the *Nyctalus* genus are a conservation priority because a high proportion of the individuals are at collision risk with wind turbines (Roemer et al., 2017)~~; to spare their populations, wind energy planning should therefore avoid areas where these species are extant.~~"

Lines 129-133: I would recommend you summarize this part. My suggestion: "The aim of our study is to assess the effects of the local habitat, coupled with bat density and movement patterns, on road collision risks." Right now it's too long and fairly repetitive, since some of the specific methods mentioned in this section are basically tackling the same goals. Also, note that I've changed landscape to habitat, since you are looking at small-scale, not large-scale, features.

➔ We thank the recommender for this suggestion. We changed this part as following: "The aim of our study was to assess the effects of the local habitat, coupled with bat density and movement patterns, on road collision risks. ~~Our study aimed at (1) assessing the effects of the local landscape on bat activity and movement behaviour and consequently on road collision risks, (2) disentangling the roles of density and movement behaviour in collision risks, (3) determining how the orientation of linear vegetation affects the orientation of bat trajectories, to provide guidance for mitigation measures and (4) providing a proxy for species susceptibility to road collisions independently of their population sizes.~~ In order to provide species-specific answers, our analyses were mostly performed at the species level, using the guild level only for species with small sample sizes. In addition, one of our goals was to provide a proxy for bat guilds susceptibility to road collisions independently of their population sizes."

Lines 140-143: I am not sure you have provided sufficient evidence as to why you'd expect bat density or the proportion of animals flying in the zone at collision risk to influence collision risk in different ways in different contexts. I think the ideal approach is to simply mention that it's expected that larger number of bats flying in the risky zone would mean greater collision risks. If not, then there may be site, habitat and/or species-specific traits that influence collision risks. Lines 143-150: I think it is now great that you have added

some predictions, but in my opinion, they still need a lot of work. Let's take it one by one: (1) a higher bat density at good quality habitats (i.e. tree rows near streams and tall trees) and at roads with a lower traffic rate. These are two separate predictions, right? Also, is it possible to generalize that having tree rows near streams and tall tress is a good habitat for all species considered? One alternative here is to simply mention that previous studies have found greater activity in sites with those type of conditions, similarly to what's mentioned in lines 92-94. Provide some evidence (perhaps a reference) that can support the second part of this prediction (more cars = fewer bats). Your second prediction, (2) a higher proportion of individuals in the zone at collision risk when vegetation grows closer to the road and in habitats with dense vegetation at each side of the road compared to habitats without trees. Are these two, again, separate predictions since they represent different explanatory variables? For your third prediction, (3) a correlation between the orientation of bat trajectories and the orientation of linear vegetation, you need a reference to support this assertion. If there are no previous studies that provide this evidence, then you need to remove this from your predictions. Overall, I don't understand the need to separate your predictions by response variable. For example, why expect that traffic affects bat density but not flying in the zone at collision risk, or that habitat configuration affects flying in the zone at collision risk but not bat density?

- ➔ We agree that this section needed some extra work. We edited it so as to follow the recommender's suggestions:
- ➔ "~~Concerning the response variables, w~~We expected bat density to be the main factor influencing collision risks in some contexts (for example, in ~~at tree rows along streams, which are rich in insects~~ habitats classified as favourable for bat foraging and commuting such as forests and riparian habitats) (Gaisler et al., 2009; Lesiński, 2007; Medinas et al., 2019, 2013), but we expected the proportion of individuals flying in the zone at collision risks to be the main factor in other contexts (especially in forested areas and when vegetation grows closer to the road, acting as a conduit (Kalcounis-Rueppell et al., 2013) and possibly forcing bats to fly over the road). ~~Concerning explicative variables, we expected (1) a higher bat density at good quality habitats (i.e. tree rows near streams and tall trees) and at roads with a lower traffic rate, (2) a higher proportion of individuals in the zone at collision risk when vegetation grows closer to the road and in habitats with dense vegetation at each side of the road compared to habitats without trees, (3)~~ In addition, we expected a correlation between the orientation of bat trajectories and the orientation of linear vegetation (Holderied, 2006; Kalcounis-Rueppell et al., 2013; Limpens and Kapteyn, 1991), ~~(4)~~ and a larger proportion of individuals flying in the zone at collision risk for short-range echolocators than for mid-range echolocators and long-range echolocators, reflecting the vertical niches of those species (Roemer et al., 2019)."

Line 172: Abbreviate genus name upon second mention (e.g. Pinus halepensis).

- ➔ We edited the manuscript according to the recommender's suggestion.

Table 1 (legend): What is the second sentence referring to?

- ➔ We apologise for this sentence that should have been deleted even before submission, it is obsolete. We deleted it.

Line 198: Change cumulated to accumulated.
Line 263: I don't understand why you mention Figure 2 here. I would suggest to remove this.

- ➔ We edited the manuscript according to the recommender's suggestions.

Figure 3: Why is the estimation of flight trajectory orientation a qualitative component of your model? I am still not entirely convinced that this estimate, flight trajectory, is adding much to our understanding of collision risk, primarily because you have not provided evidence from previous studies that either a parallel or perpendicular trajectory entails greater risks.

- ➔ We agree that this part needs an additional explanation. We added it l. 345-351:
- ➔ "This model is not a quantitative estimation of the collision risk at a road section, since the road sections that we studied were approximatively squared, and thus the orientation of bat trajectories does not influence the time spent at risk of collision. This model was therefore made to provide a qualitative estimation of the collision risk that can help the design of mitigation measures. Even if bats flying parallel and above the

road do fly for a longer among of time at risk of collision than bats crossing roads, in the case of our study, we assessed collision risks relatively to a road section and not relatively to a bat individual."

➔ We also agree that the need of studying trajectory orientation was not properly described in the introduction, and we edited the manuscript accordingly (l.110-115):

➔ "But they did not take into account bats flying parallel to the road axis, although this behaviour may be a determinant factor of collisions, because ~~in this situation bats spend much more time in the zone at collision risk.~~ bats flying parallel to the road axis may fly at risk of collisions for dozens of meters, while crossing a road only implies flying at risk of collision for a few meters. Mitigation measures to reduce collisions are also mainly designed for bats crossing roads (Elmeros et al., 2016), although it is not known to which extent bats may follow the road axis or cross roads, depending on the habitat context."

Line 314: Change "to fly" to flying.

➔ We edited the manuscript according to the recommender's suggestion.

Line 315: Is this estimate including flights only at a vehicle height or does this include at vehicle height on the road?

➔ Indeed, we did not mention that this estimate includes flights at vehicle height on the road. We completed the sentence: "The probability of trajectories to be in the zone at risk (i.e. at vehicle height and above the road) was modelled using the risk status of each trajectory as the binomial response variable (0 = safe; 1 = unsafe) (Figure 2)."

Lines 326-328: I am still not convinced that you can safely assume this (still seems speculative), so I recommend that you delete this sentence.

➔ We edited the manuscript according to the recommender's suggestion.

Lines 329-332: This is still something that must be addressed carefully, mainly in terms of providing evidence that a parallel, or perpendicular, flight path orientation entails greater collision risks.

➔ We kindly refer to our edition of the manuscript, as proposed above:

➔ l. 345-351:
"This model is not a quantitative estimation of the collision risk at a road section, since the road sections that we studied were approximatively squared, and thus the orientation of bat trajectories does not influence the time spent at risk of collision. This model was therefore made to provide a qualitative estimation of the collision risk that can help the design of mitigation measures. Even if bats flying parallel and above the road do fly for a longer among of time at risk of collision than bats crossing roads, in the case of our study, we assessed collision risks relatively to a road section and not relatively to a bat individual."

Lines 362-363: Can you please explain this a bit better? I am not sure first why you did this, or how. Why do you need your explanatory variables to follow a normal distribution? How did you normalize them?

➔ Normalising the distribution of explanatory variables is not absolutely required to model the response variable with a GLMM, however to avoid potential problems with outliers, we normalised the distribution of the explanatory variables that were heavily skewed. In this case we used the square root function. We edited the manuscript to better explain our method:

➔ L. 383-384: "All descriptive variables were normalised if necessary and scaled to follow a normal distribution and to compare their effects. Thus, distance to vegetation and traffic were normalised using the square root function."

Line 364: remove the error message. Line 394: Change problem to problems.

➔ We edited the manuscript according to the recommender's suggestion.

Lines 394-397: What was done with the models that did not converge?

➔ We agree that this part needs a clarification, we added this sentence:

➔ L.418-419: "When model convergence failed, the model could not be built and was not considered for selection."

Legend figure 4: Please arrange the order of the x-axis categories in the same order as those displayed in the graphs (e.g. first F, then FE, DPT, etc.).

➔ We edited the manuscript according to the recommender's suggestion.

Line 401: Landscape (habitat?) type refers to which variables in table 2? Perhaps what is needed is a new main column in table 2 called habitat/landscape type and then subdivide it with pertinent categories.

➔ We edited the manuscript according to the recommender's suggestion.

Table 2: Please switch columns and rows. Your columns should be the explanatory variables, and rows should be species. The same applies to tables 3 to 5.

➔ We understand that the presentation of results in tables 2 to 5 can be overwhelming, and we put a lot of effort in the choice of the layout. Our impression is that the large majority of scientific papers that we read put the explanatory variables as rows and species as columns (e.g. Azam et al., 2018; Kitzes and Merenlender, 2014; Lewanzik et al., 2019). We thus think that it would lead to confusions to do otherwise.
➔ If it is possible, we would thus prefer to keep the tables 2 to 5 as they are.

Line 442: I believe some results should be provided to understand this a bit better, so please remove "results not shown" and provide more evidence for your assertion. At the moment the only way to understand flight trajectories is to look at the estimate of the model in table 5, which is not that straightforward.

➔ We agree and we provide the graphical representation of the orientation of flight trajectories as supplementary material (Figure A3).

Lines 457-460: It is normally not widely accepted that paragraphs are composed of a single sentence. In this case, I believe these two ideas should be merged into a single paragraph. Line 500: change to explanatory variables.

➔ We edited the manuscript according to the recommender's suggestion.

Lines 507-508: you need a reference here.

➔ Indeed, we thus added it according to the recommender's suggestion:
➔ It is rather unlikely that the structural composition (density and orientation of linear vegetation) is the only explanation for these differences, because other confounding effects may very well increase bat density also, such as the different tree species that were often associated with a type of landscape; for example, it is known that *P. pygmaeus* prefers riparian habitats (Rachwald et al., 2016).

Lines 518-522: My opinion is that you do not need to explain all of your results, especially if there is no way to explain them without resorting to speculation. For example, you mention that there is higher social activity in N. leisleri, but you have provided no evidence for this. I'd suggest you remove this entire sentence.

➔ We edited the manuscript according to the recommender's suggestion.

Line 547: The word "avoided" implies that bats actively reduce their activity in the zone at risk when they perceive traffic, which you are not providing evidence for. I also do not agree with lines 550-551: "These results show that bats spatially avoid the vicinity of vehicles". I guess you'd need a similar set of data as that obtained by Zurcher et al. (2010), where you can clearly see that bats change their course when cars are detected, to explain a reduction of a species' activity at the zone at collision risk during higher traffic. Some of the speculation in this paragraph continues in the next one, particularly in line 558 ("more reluctant to approach vehicles") and line 563 ("their vehicle avoidance behaviour").

- ➔ We agree that there was unnecessary speculation in these parts and we edited the manuscript to be more factual:
- ➔ L. 570-572: "Nonetheless, P. kuhlii/nathusii ~~avoided~~ flew less in the zone at collision risk ~~more~~ when traffic increased, possibly because they recognise the danger associated with vehicles, although a specific data set would be required to test this hypothesis."
- ➔ L. 573-575: These results ~~show that bats spatially avoid the vicinity of vehicles,~~ complete~~ing~~ the observations of Zurcher et al. (2010),~~. The latter~~who did not distinguish between species, but found that 60% of approaching individuals reversed their course in the presence of a vehicle.
- ➔ L. 580-584: "~~It could be that because SRE fly lower and are more at risk when crossing (Roemer et al., 2017), they are more reluctant to approach vehicles. In addition, s~~ Since the foraging abilities of SRE seem to be more impaired by light and noise than MRE (Azam et al., 2018; Siemers and Schaub, 2011; Stone et al., 2015), MRE might use roads as foraging grounds and take more risks than SRE."
- ➔ L. 584-587: "Therefore, even if SRE are known to fly lower than MRE and thus at heights more similar to those of vehicles (Berthinussen and Altringham, 2012; Roemer et al., 2019), their lower bat-vehicle ~~avoidance behaviour~~ co-occurrence should partially mitigate their susceptibility to collisions."

Lines 567-575: You are focusing this section on the more speculative ideas rather than those for which you might have greater support from previous studies (and your own data). Why is there an increase in flights at the zone of risk during the summer, for example? You did not explain this in the discussion. This increase may simply be the result of an increase in bat density during the summer (which you clearly show in your data), and not necessarily because there is a larger proportion of those flights occurring in the zone at risk (implying riskier behaviors or greater naïveté). The only two species for which I believe you have sufficient evidence of an increase in the number of bat passes at risk per night as the years progresses (and greater during the autumn compared to the summer) are P. pygmaeus and Plecotus sp. For those two species, the number of bat passes at risk per night may increase due to an overall increase in bat density and/or a larger proportion of flights occurring at the zone of risk. Without additional data, such as whether those flights at risk are predominantly juvenile, or evidence from other species which also have volant juveniles during autumn, the section about juveniles and sexual activity driving risky behaviors is speculative.

- ➔ We agree that some sentences can be edited to reduce speculation.
- ➔ We also completely agree that the increase in the number of flights at collision risk is highly supported by the increase in bat density, and we edited the manuscript to make that clear:
- ➔ L. 590-594: "Our results show typical activity patterns throughout the year with peak density in summer or autumn, that seemingly drive the number of bat passes at collision risk per night (the product of quantitative models), that also shows a peak in summer or autumn. ~~but~~ However, it is the first time to our knowledge that it is demonstrated that flight proportion in the zone at risk at roads increases in autumn (for ~~most~~ several species and ~~all~~ guilds)."
- ➔ Concerning juveniles, we agree that we did not provide enough convincing arguments. We edited the manuscript accordingly and removed unnecessary speculation:
- ➔ L. 594-600: "An increased flight proportion in the zone at risk in autumn could partly be attributed to the naïve behaviour of juveniles, ~~not aware of the danger of road vehicles~~ which after birth and emancipation suddenly increase population sizes at the end of the summer (Dietz et al., 2009), and that are necessarily present in our dataset even if we cannot assess their proportion. Juveniles are indeed more vulnerable to road collisions than adults (Fensome and Mathews, 2016). This result could also be explained by increasing foraging opportunities on roads during colder times, as was observed in swallows (Evans et al., 2003), and increased energetic demands before hibernation (Dietz et al., 2009). ~~Bats may also be less cautious at this time of year where they are sexually active (i.e. increases in sexual hormones may decrease awareness of potential danger (Alcock, 2009)), and where they need to fatten before hibernation.~~"

Lines 627-628: I believe the aims of your study can be attained (at least approximated) with direct counts of carcasses, yet with a significantly greater investment of time. Maybe this would be worth mentioning, adding a reference to studies that have counted carcasses to asses risk (e.g. Medinas et al. 2020: https://doi.org/10.1016/j.jenvman.2020.111412).

- ➔ We agree and we edited the sentence as following: "These results underline ~~the need of using acoustic recordings to collect enough data per species~~ the fact that to attain the aims of our study and to collect enough data per species with direct counts of bat carcasses, it would have been necessary to invest a significantly greater amount of time than it was necessary using acoustic recordings."

Lines 633-635: add a reference to this sentence. Line 651: change to "habitat loss for numerous". Lines 665-666: Remove authors from parenthesis.

➔ We edited the manuscript according to the recommender's suggestion.

I thank you for your continuous effort to improve this wonderful preprint, and hope to see it finalized soon.

Kind regards,

Gloriana Chaverri

Azam, C., Le Viol, I., Bas, Y., Zissis, G., Vernet, A., Julien, J.-F., Kerbiriou, C., 2018. Evidence for distance and illuminance thresholds in the effects of artificial lighting on bat activity. Landsc. Urban Plan. 175, 123–135.

Kitzes, J., Merenlender, A., 2014. Large roads reduce bat activity across multiple species. PloS One 9, e96341.

Lewanzik, D., Sundaramurthy, A.K., Goerlitz, H.R., 2019. Insectivorous bats integrate social information about species identity, conspecific activity and prey abundance to estimate cost–benefit ratio of interactions. J. Anim. Ecol. 88, 1462–1473.