

Reviewer 1 (Lia Gilmour) **comments back in red**

Basic reporting

Please see some comments in the line by line comments below on basic reporting. I have some criticisms about the way the methods are reported in general. This section could be made much more concise and headings would be helpful. The schematic is useful. There are some missing bits or ways it could be restructured so it is easy to pick out salient information.

Line by line comments. Please refer to the annotated review of the pdf for these comments and highlighted areas.

L100: Include Gilmour et al. 2021 <https://doi.org/10.1242/jeb.242715> Jamming hypothesis is perhaps a bit simplified understanding now of how bats respond to UD.

We added, "Acoustic deterrence has been demonstrated to decrease bat activity, foraging behavior, and flight performance, potentially due to auditory masking that precludes the use of echolocation (Gilmour et al., 2021)."

Track Change line 104

No Track Changes line 103

LG response: great, thank you.

L112: Again, you fail to include the work done flight path tracking bats in response to UD Gilmour et al 2021. Only one species tested, but a start in this area of work.

This paragraph is focused specifically on using UD to reduce bats at wind energy facilities; thus, this citation is not included here.

LG response: agreed, no need to change.

L128 This section could be made more concise and structured better. E.g. combine the fact that you are placing two UD in the flight cage into the first paragraph and add details later. Otherwise you are sent to look at Figure 1 before you have the full info on experimental set up.

We added "The open-air flight cage was approximately 60 m x 10 m x 4.4 m (length x width x height), was surrounded by 6.4-mm, lightweight, plastic netting (Industrial Netting, Minneapolis, Minnesota, USA), and with a UD mounted on a pole ~ 1.5 m high on each end."

Track Change line 140

No Track Changes line 139

LG response: great, thank you.

L128 Perhaps the addition of headers for the different methods sections would make this an easier and more organised read? If the journal allows?

We restructured this section for clarity and do not believe subheadings are needed.

LG response: agreed, no need to change.

L129 Over which dates, on how many nights? Multiple years. This is very important info. Temp and humidity controlled for or collected? Rainfall nights excluded? This section needs significant improvement and clarification.

We did not control for temp or humidity as trials were in an outdoor flight cage built specifically to assess bat responses to deterrents in real-world weather situations. We did not examine the influence of weather because, again, bats would experience similar variance in weather conditions at a wind energy facility and our focus was on the broad application of UD's. So, although temp/humidity play a role in the attenuation of sound, its not something that we can control in the real world (i.e., bats will experience similar temp/humidity changes at a wind energy facility). So, for deterrents to be effective bats essentially need to respond to the signal across a range of conditions. Dates are included in specifics 2 paragraphs later, but we added the specifics dates of the seasons to this section for clarity. We did not conduct trials in the rain.

LG response: good explanation of why not needed, but maybe one line saying this would be helpful?

We added, "We captured bats within 120 km of the flight cage (typically within 30 minutes in Hays County, Texas, USA) on both public and private properties for which we had authorization using mist nets, harp traps, and hand captures from July 13, 2020-October 7, 2020 for the fall season (45 trial days) and March 5– May 15, 2021 (40 trial days) for the spring season."

Track Change line 169

No Track Changes line 166

LG response: great, thank you.

L140: State that the UD's were at either end of the flight cage, as in the figure.

We added, "The open-air flight cage was approximately 60 m x 10 m x 4.4 m (length x width x height), was surrounded by 6.4-mm, lightweight, plastic netting (Industrial Netting, Minneapolis, Minnesota, USA), and with a UD mounted on a pole ~ 1.5 m high on each end."

Track Change line 140

No Track Changes line 139

LG response: great, thank you.

L142 Why these specific frequencies? Were they chosen as you state in next paragraph? If so why step up by 6 kHz each time, some explanation would be helpful.

These frequencies were programmed on the NRG Systems deterrent and was the configuration of the commercially-available UD at the time of the study. We added this information to the manuscript.

Track Change line 154

No Track Changes line 152

LG response: great, thank you..

L143: Across frequencies?

I'm not sure exactly what this is referring to, but we changed the paragraph for clarity.

LG response: great, thank you..

L144: Can you calculate a dB table for the F bands at a range of distances from the UD's? This may have a bearing on how a bat responds to the different F bands, as higher bands may attenuate more quickly, so the bat is not experiencing a uniform SPL for all bands.

Thank you for the suggestion. This calculation has been completed before (see Weaver et al. 2020) and was acknowledged in the discussion (line 294 in original submission).

LG response: great, thank you..

L49: missing 'was'. Also this sentence maybe should be combined with the last, or include "for this species"?

We made this change.

Track Change line 49

No Track Changes line 49

LG response: great, thank you..

L160 Two hours driving? A distance would be better in km?

We made this change.

Track Change line 169

No Track Changes line 166

LG response: great, thank you..

L162 This info needs to come earlier and an indication of how many nights the experiment took place over. It all needs a bit of a restructure, so key info is covered earlier about experiments and details come later.

We restructured this section.

Track Change line 171

No Track Changes line 168

LG response: great, thank you..

L63: human-induced?

We changed to "anthropogenic"

Track Change line 64

No Track Changes line 63

LG response: great, thank you..

L164 What was the reason for feeding the bats?

Because we held bats for several hours and prevented foraging. We added this information.

Track Change line 175

No Track Changes line 171

LG response: great, thank you..

L165 Include the length of the different periods in the text as well as in the figure.

We clarified this text and added this information.

Track Change line 177

No Track Changes line 174

LG response: great, thank you..

Also have you got any reasoning on the acclimation period length, or something to reference on this. It seems very short, but there may be a reason for this.

There is not robust documentation regarding the amount of time the acclimation period should be for this type of study. Most bats were flying freely once released; thus, this amount of time seemed reasonable. We felt that we did not want to add stress to a bat by being in a flight cage, but wanted to be sure it was aware of the flying area. Based on the videos, 4 minutes seemed to do that. Additionally, we had a preliminary season in Fall 2019 that we do not include here where we ensured the robustness of our field methods.

LG response: great, thank you, could you mention something about the preliminary season maybe to make this clear?

L171 Why? Include a reasoning for this.

We made this change. We did this to limit any potential bias from a bat preferring one side of the cage for an unrelated issue and if bats did not fly during the trial.

Track Change line 184

No Track Changes line 181

LG response: great, thank you..

L172 Good, but again why? Include a reasoning for this.

See above. We omitted some information for clarity.

L178 What protocols?

See below.

L177 Can you be clearer here- why were bats injured? During capture, or in the experiment. This worries me. Were there any severely injured bats? If so, how many? This probably needs rewording, as sounds convoluted and unclear. Was it just you had permission to do this, had there been an injured bat, or you did have to do it?

We omitted this section for clarity. This statement is not required for PeerJ. We included our state and IACUC permit information.

LG response: great, thank you..

L198 Did cameras have any distortion at edges? Was this accounted for?

We accounted for distortion within the code. We added this information.

Track Change line 214

No Track Changes line 204

LG response: great, thank you..

L206 What was your reasoning for not using a GLMM or something similar? It seems that you may have an order affect of your C vs T periods, i.e. bats could become habituated, or some other order effect related to flight cage stress, or stress after handling.

Would it not make sense to use a random effects model?

We clarified our reasoning for quantile regression including the large number of observations per bat and because quantile regression allowed us to detect differences when they did not occur homogeneously across distances, which would be missed by regression models. We added this information.

Track Change line 223

No Track Changes line 214

LG response: great, thank you..

Or this? <https://cran.r-project.org/web/packages/lqmm/lqmm.pdf>

I am really not convinced with your stats method. You will have to explain how you accounted for multiple comparisons and why you did not consider including treatment order as a random effect. *We updated some of the writing for the methodologies for clarity. Treatment order was random so is accounted for. We used this stats method because we have locations of bats 30 times per second x 4-min treatments (3) x 4-minute controls. These locations are autocorrelated due to the small time between frames. This method basically uses bat ID similar to a random effect.*

We attempted the using the lqmm package for linear quantile mixed-effects modeling; however we have had problems using this package and did estimates that seem reasonable. We consulted a statistician (Brian Cade) who has had a number of email exchanges with the developer of lqmm over the years but have never had satisfactory explanations of what needs to be done to get reasonable, reliable estimates from this lqmm package. Using lqmm was very slow to converge to a solution for a single tau and was not consistent.

LG response: great, thank you, I am convinced you have explored all methods fully and thank you for your detailed explanation. Great to get advice of a statistician too, that's excellent to hear.

L208 Would you not need to include a correction for multiple comparisons here, e.g. Bonferroni or something similar?

A correction is not needed when assessing multiple quantiles with quantile regression. However, we can apply a Bonferroni to the treatment differences, which is now in the table legends and methods.

Track Change line 244

No Track Changes line 234

LG response: great, thank you..

L214 Did you compare just the first control period to the first treatment period, or to all treatment periods combined?

We first compared all controls and control periods did not differ. (Line 213-214). Thus, we selected only the first control to compare to all treatments so that sample sizes would be similar. If not, we would have a maximum of 7,200 points (4 minutes with 30 frames per second) for the treatment periods and 21,600 points for all controls combined.

LG response: great, thank you..

L220 Again, if you are doing multiple comparisons, you should really include a correction for this, as you will increase Type 1 error (false positives) the more tests you do.

We only compared one treatment to one control at a time due to the extremely large sample sizes. Please see above for Bonferonni info.

LG response: great, thank you.

You could also include all of these fixed effects and interactions in one big model, surely that would give you better power to detect effects and make more sense statistically?

Due to different sample sizes, the extremely large datasets, etc., these models would not converge.

LG response: great, thank you, you have answered above.

L237 There has got to be a better way of presenting the data. These plots are hard to interpret and compare between species. A box plot with all species responses to each F band would be much better and maybe the sex and season \ info represented in a table or something? Or if you can get the sex and species in there too that would be great, if it wasn't too busy. I appreciate its difficult to tease apart, but this definitely needs work. The whole premise of the paper is comparing species-specific differences, but your analysis and presentation of the results doesn't seem to follow this.

This is incorrect. The premise of the paper is not to compare differences among species (that is not what we did statistically either). Instead, the premise was to assess how multiple species responded to the three treatments because we wanted to know if a single treatment was successful for multiple species. In our opinion, it was not as useful to compare, for example, red bats to evening bats, but to know if one frequency emission worked best for both for real-world applicability.

LG response: OK, thank you for your explanation, that makes sense.

L271 Discussion: A better way to structure this would be to include headings, maybe questions linked to key findings? E.g. Do bat species differ in their responses to UDs at distance? Or something similar...

We restructured much of the manuscript and would prefer not to include subheadings.

LG response: OK thank you, if that is what you prefer.

L274 typo experimental

We made this change.

Track Change line 316

No Track Changes line 299

LG response: Great, thank you

L273 be specific, shifting how? More precise language needed

We added "away from the UD"

Track Change line 315

No Track Changes line 299

LG response: Great, thank you

L276 Maybe due to the analysis?

Due to individual variation.

LG response: Great, thank you

L296 This phrasing tends to suggest the deterrent has active part in "interacting" with individuals.

This needs rephrasing. Also better to use individuals rather than species as the responding party. The attenuation of sound with increasing distance could be explained better. This is also dependent on atmospheric pressure and temperature. Were these considered for different nights of experiments?

We changed this sentence to, "Deterrent signals that include lower-frequency ultrasound travel farther from the source and may be detected by bats at greater distances."

Track Change line 336

No Track Changes line 321

LG response: Great, thank you

I cannot see any mention of temp/humidity effects. At higher Fs humidity and temp can have a big effect on deterrent propagation over distance. This could have affected what the bats were experiencing. If the temp and humidity were fairly consistent across the experiment state so. *We included the months of the project trials. No, temperature and humidity were not constant over the project period. We did not test this specifically, but understood this would/could happen. Our main goal was to test what bats would experience from a wind turbine, in which temperature and humidity also would consistently change.*

LG response: OK, maybe best to include something that explains this?

L298 It may not just be the echolocation peak F that determines how bats respond to different F bands from UD. The call type should also be considered. Myotis bats tend to have a broader sweeping FM component compared to your "lower-frequency" bats. They therefore have in a sense, perhaps no where to go in changing their call structure in response to sound. Bats from species with a quasi CF part of their call, or other call structures may be able to modify their calls in response to a masking or "jamming" sound. See Gilmour et al 2021 again. This is crucial in understanding why bat species may respond differently to UD F bands. This needs more discussion. Reference to low or high F bats is too simplistic, as call structure and duration may also play a part in species-specific responses.

I am not familiar with the call structures of these bats, but it may be worth considering as a line of reasoning!

Thank you for this suggestion, we agree. In this paper, we focus on how bats actually respond to the UD, regardless of the mechanism responsible for said response. However, we will have a companion paper that focuses on echolocation in greater depth.

LG response: Great- looking forward to reading that one!

L301 Again a heading would really help here to break up the discussion and make it clearer what we are discussing.

We prefer keeping headings out of the discussion and hope the restructuring prevents the need for headings.

LG response: no problem.

L302 Any suggestions to why there may be a difference in responses of the sexes?

How do they differ? In what direction? This is too vague.

Why sexes may respond differently would be purely speculative. There could be a behavioral or physiological component, but there is not research that we know of to support either at this time. The differences are in the figures. We have a supplemental table we could include, but it includes a lot of information.

LG response: OK, thanks for your response

L329 So why could there be a difference here between season and species? Maybe some of these things going on here too. Link back to your results.

Again, any differences would be purely speculative. Further research needs to be conducted to understand the potential physiological or behavioral reasons for differences, which were outside the scope of this manuscript.

LG response: OK, thanks for your response

L338 This is an important point

Thank you!

L347 Interesting!

Thank you!

L350 Try not to spend too many words explaining the limitations. This bit can be cut down a bit as it reads more like a thesis or report than a paper. These bits could be incorporated into the methods somehow or cut down to a short concise paragraph. They are important points, but you have good reasoning to do it the way you did, so discussing limitations at the end reduces the impact of the study.

We cut this paragraph down. Thank you.

LG response: great

L362 And Gilmour et al. 2021

We added this citation.

Track Change line 407

No Track Changes line 390

LG response: great, thank you

L387 This sentence needs to be reworded. "Shift activity" seems the wrong phrasing, since you have been using distance all the way through.

We changed this to "This study demonstrated that certain bat species respond to different ultrasonic treatments, but variability among species existed."

Track Change line 445

No Track Changes line 409

LG response: great, thank you

L385 This should just contain a punchy outline of the main conclusions. I would suggest removing the second paragraph and any limitation suggestions into the main discussion and keeping it concise and a confident round off of why your study is great, because it is! I feel you are not selling it enough.

Thank you. We revised as suggested.

LG response: great, thank you

L390 Again focusing on call type rather than species would be a better way to approach this study. There are standard ways of doing this in the literature. This would make it more applicable to international audiences looking at bats that have similar acoustic niches to those tested in your study.

Thank you, we agree; however, this was not within the scope of this manuscript. Herein, we focus on the flight response to the UD's, which has a management-oriented application regardless of the underlying mechanism. However, in subsequent work, we plan to focus on potential mechanisms for these behaviors.

LG response: OK thanks for your explanation and looking forward to that follow on work

L393 What's the point in the study then? You could reword this, saying something like "caution must be taken extrapolating these results to a wind turbine application" or "future studies should focus on how these \ results compare to a wind turbine in situ..." or something along those lines! This is a proof-of-concept for the overall application to WE scenarios, looking at specific behaviour. It's always going to be hard to extrapolate. But this phrase seems to suggest the experiment is pointless!

Thank you for this important suggestion. We changed the phrasing as you suggested.

Track Change line 445

No Track Changes line 409

LG response: great, thank you

L398 Explain why... this might be better in the main discussion

We explain this in the subsequent sentence, “Complex signals may further disorient bats who might adapt to constant stimuli” – however this is speculation and needs further investigation.

LG response: noted, thank you

Experimental design

While I have no problems with the experimental design in its basic form, the analysis performed needs some work, or more explanation. Please see my detailed comments in the line by line section.

We clarified and reworked the section.

Validity of the findings

Some work needs to be done to convince me of the findings. They also need to be presented more clearly and reanalysis may make this possible. If the results could be presented and discussed so they are applicable to a wider audience this would make the work much more accessible and the impact higher.

We clarified throughout.

Additional comments

This is an important study in the field of deterrent research and seems to be a really good flight cage experiment, well thought out and designed, within the limits of possibilities. It is an important next step in understanding how ultrasonic devices can be used to reduce mortalities of bats at wind turbines. The manuscript however is lacking detail in places and a strong message concluded from the results is missing. I recommend an alternative analysis method and a better way of presenting and discussing results. These corrections would really improve the manuscript and significantly increase its impact in the field. It also needs some work making it applicable outside of the north American scenario. If bats were described based on their call characteristics or in a guild specific way perhaps, it would be easy to compare to European bats or those elsewhere where wind energy is expanding (India for example).

Thank you. We will focus on the echolocation behavior in a corresponding manuscript.

I have strong reservations about the statistics and the way the analysis has been performed, especially with multiple comparisons and no correction applied (or reference to if it was applied). Major re-writing needs to take place and perhaps some reanalysis. But if this is done, I

wholeheartedly would like to see this published. I can not recommend for publication without significant corrections.

Corrections are typically not used in quantile regression when comparing multiple quantiles. We did, however, apply a Bonferroni to the treatment level differences, which we added language to. We consulted with experts across multiple statistical fields with Brian Cade assisting in analyses. We believe this statistical method to be the most robust for the data structure.

Extra comments from LG on second review:

The methods and discussion sections are very well structured now and although I would prefer headings throughout, I can see that the work done has made it much clearer to read and find the necessary information. Everything is clarified and I am satisfied that the authors have made a substantial effort based on my suggestions. I was particularly impressed by the explanation on the statistics, which I have reservations about. I am happy now that they have exhausted all possibilities and come up with the best methodology to gain the insights they were hoping for from the study. This is a brilliant study and I would be happy to see it published.

General comments

A few minor suggestions and responses to the comments, however I do not need to see again and will leave to the authors discretion. There are a number of typos due to track changes that we suggest the authors check before final publication. I may not have found them all.

Line 150: two words together, sure you would find that in the final edit, but just flagging for ease.

Line 167: two words together

Line 409: extra space and period