This study addresses a significant gap in the literature by investigating microplastic exposure in North American insectivorous bats, a topic that has not been previously explored. The methods used are reliable and adapted from established protocols. They found a negative association between MP concentration and bat body mass, suggesting potential health consequences. Generally, the manuscript is well-structured and straight forward. This study addresses an important topic and makes a valuable contribution to the field. However, certain aspects of the manuscript need further refinement to enhance clarity and strengthen the argument. With revisions to improve clarity of presentation and depth of the discussion, the paper will be well-positioned for publication.

General comments about methods: Procedural blanks are used to calculate limits of detection (LOD) and quantification (LOQ) e.g., https://doi.org/10.1016/j.jhazmat.2022.130218 and https://doi.org/10.1021/acs.est.0c03211. LOD can be used to calculate the incidence of ingestion. Based on your blanks, your LOD is 8 MP, meaning 65.4% of the bats had MPs in their GITs. Since your LOQ is 20 MP, only bats with 20 or more MPs should be included in concentration calculations and analyses with body mass. You could partially address this by making statistical comparisons with your blanks, but it would be helpful to discuss the reasoning behind your approach and why standard LOD and LOQ protocols were not followed.

General comments about discussion: Your bats are not a random sample, rather they are a sample of convenience which can introduce bias. I do not think this is much of a problem here, but it should be addressed in your discussion along with potential sampling alternatives, like using emetics or lethal sampling.

Line by line comments are below:

Lines 20-24: As with much of the manuscript the writing is clunky, in that sentences are blunt with a lack of transition between thoughts. Sentence structure should be more varied.

Lines 21-22: Not all bats are insectivorous, and this sentence should be revised to accurately reflect this fact.

Lines 25-27: Little brown bats were used to develop the methods and were not used in any of the data analysis. The phrasing here is therefore misleading.

Lines 46-48: The sentence starting with “Additionally” is awkward and confusing.

Lines 58-61: Citations are needed for the threats to bats.

Line 66: The whole section headed by “Bat stomach content collection”: This section is about getting samples to develop the methods used for the “real” analysis. Perhaps a better heading would be “collection of samples for protocol development”? As it is written it is awkward and misleading.

Lines 71-72: How else might samples be collected if not by hand? By drones or robots? If the method is uncertain, it may be best to acknowledge that directly to avoid raising unnecessary questions for the reader.

Lines 80-81: Could you clarify whether any of these bats died from WNS? What percentage were missing brains? I assume that bats with intact brains likely did not die from encounters with people or pets—was this considered? Additionally, if bats without brains were included in the body condition analysis, how was this accounted for? Providing these details would help strengthen the interpretation of the findings.

Lines 85-87: You mention that methods minimized risk of MP contamination. Was all equipment used in necropsy rinsed 2-3X with DI water? Or did you rely on rinsing the GIT with DI water to minimize MP contamination?

Lines 94-95: The authors do not clearly explain how the little brown bat stomach contents were used in protocol development. It may be helpful to discuss this separately in the methods section or consider removing references to the little brown bats altogether. Since the method testing with bat stomach contents does not significantly contribute to the key findings on large brown bats, clarifying or streamlining this aspect could improve the focus of the paper.

Line 110: typo “wo”

Line 123: Link does not work

Line 131: Did you use any of the suggested improvements? If not, then this sentence would be better placed in the discussion section and explored as a potential limitation of the study

Line 134-135: What were these pictures used for? No measurements were presented in the results. Clarification needed here.

Line 139: “mass of KOH solution” – are your data the mass of the KOH solution or just the weight of KOH used to make the 10% solution? If it is the mass of the solution, then you used very little of it. Looking at the data, all blanks were less than 3mL in volume? And GIT could be completely submerged in 1.5 – 8.8 mL of KOH solution? If you used such small volumes, then how did you make the 10% KOH? Did you make a 10X stock solution that you diluted for the procedure? If so, was it filtered before use? How did you ensure that the mass of your sample plus the KOH was accurately weighed to 0.0001g, for example your 11.7088g sample (EPFU_57_GI)? Did you use an analytical balance that was accurate to 0.0001 grams? Clarification needed here.

Line 140: You mention that you log transformed the data. This suggests that your data are positively skewed, yet you report means in your tables. Are these back transformed means? If not, then you should report the medians as well. Instead of transforming your data, you could use a GLZ analysis with a gamma distribution and a log link function. This allows inference about the arithmetic mean on the original scale.

Lines: 148-154: Not sure this is needed (see comments for line 94-95 above)

Lines: 161-162: Here are the measurements, I missed the measurements in my first read through. They do not seem relevant, especially if you lump all sizes into one MP group. I am curious about how many (%) were 1-5mm? Do your results change if you only use 1-5mm sizes?

Line 172: your p-value is marginal, please report your effect size. You should also report your test statistic and degrees of freedom.

Line 172-174: If the null hypothesis is not rejected, it is important to avoid stating that one value is higher, as no significant difference is detected. Given the small sample sizes, you might also consider reporting your statistical power.

Line 178 & Figure 4: consider adding sample sizes for each month in the figure instead of in the text. This can explain the lack, or large size, of your error bars. You should explain what your error bars are in the figure caption.

Lines 181-184: To make claims about something being the most, highest, or lowest, statistical analysis is needed. You can describe trends, but it is best to do so in the discussion section rather than in the results.

Line 186: In scientific writing, it is best to avoid the word "proven," as research typically supports, suggests, or provides evidence for a conclusion rather than definitively proving it.

Line 199: The phrase "free-ranging microplastics" seems a bit unusual, as microplastics are generally dispersed in the environment rather than confined. Consider using a more precise term, such as "environmental microplastics" or "circulating microplastics," to clarify the intended meaning or removing the descriptive entirely.

Line 234: You have your r^2 value, so you know exactly how much variability in bat mass you explained with your model (23%).

Lines 235 – 254: This section could be streamlined for clarity, as some parts are somewhat repetitive and the sentences are quite long and complex. It may benefit from a bit more refinement. Also, a period is missing at the end of the last sentence.