12-Aug-2021  
  
Dear Dr. Hardy,  
  
Thank you for submitting your manuscript "Quantifying wildlife conflicts by combining eDNA metabarcoding and traditional diet analysis" (21-372) to Conservation Biology. I have received a thorough, constructive review. The review is pasted below.  
  
Normally, we require 2 or 3 reviews. However, the recruitment of reviewers at this time of year has been slow. The review in hand is thoughtful and recommends 'reject-and-resubmit', raising significant concerns about the methods. Rather than delay things further, on the basis of the review and recommendation, I will decline the manuscript at this time, to give you time to consider these comments and, potentially, to revise accordingly. I will consider a resubmission that fully addresses the concerns described below.   
  
Resubmitting your manuscript does not guarantee eventual acceptance, and your resubmission will be subject to the full peer-review process. You have 90 days from the date of this letter to resubmit. If you anticipate resubmitting beyond that date, please contact me.  
  
To resubmit the manuscript, log into your Author Center at <https://mc.manuscriptcentral.com/conbio>. Click on "Manuscripts with Decisions" and then "Create a Resubmission," which is located next to the manuscript number. Then, follow the steps indicated. In the space provided or as a separate Word document, please include a detailed, point-by-point response to the comments of the handling editor and reviewers. Describe the changes you made to the original manuscript and, if applicable, explain why you did not address certain comments. If you have a Cover Letter, upload it as a separate document.  
  
Thank you.  
  
Sincerely,  
Mark Burgman  
Conservation Biology  
  
  
REVIEWER COMMENTS  
  
Reviewer: 1  
Comments to the Author  
  
Interactions and conflicts between protected/valued species are interesting research topics and have implications for conservation planning. This study provides empirical evidence for predation between two such species, and the use of fecal DNA for quantitative estimation of the predation impact is relatively novel. However, I have major concerns about the technical treatments in this study (see detailed comments below), which should be carefully addressed for the results and conclusions to be credible.    
  
1. Although both morphological analysis and molecular diet analysis showed similar detection rates for seabirds, they detected seabirds in largely different samples (only 1/2–1/3 overlap). This low consistency between the results is alarming. For samples with visible prey parts (very good DNA source), it is baffling that the DNA analysis failed to pick the signal up. There could be important improvement to the DNA workflow to increase the detection sensitivity hence its reliability. For example, were the fecal samples thoroughly mixed and subsampled from multiple locations per pellet? Multiple independent PCR replicates are also necessary to increase detection probabilities. It was mentioned that duplicate PCRs were conducted, but I saw no further report in data processing and the results regarding the duplicates.  
  
2. During dietary sequence processing, sequences were clustered based on >97% similarity (L.180). One would expect haplotypes with <3% differences (= 7 nucleotides for a ~230 bp fragment) from each other to be collapsed into one sequence following this step. It would then be impossible to analyze haplotype diversity of the penguins in the samples. However, Fig. 5 showed 7 haplotypes, 6 of which (haplotypes 1-6) had < 5 nucleotides differences and should have been collapsed into one during sequencing processing. This is quite confusing.  
  
3. Another issue with haplotype assignments. Were all haplotypes recovered from fecal DNA also found in tissue samples from the local penguin populations, i.e., were they all true haplotypes or possibly errors generated during PCR and Illumina sequencing? Artificial errors are common in DNA metabarcoding, and there should be measures to specifically identify and eliminate them to ensure data credibility. Multiple PCR replicates, sequence filtering procedures, and statistical methods can all help to reduce errors (see Tsuji et al., 2020 Mol Ecol Resour 20:1248-1258 and Tsuji et al., 2020 Environ DNA 2:42-52). It would also be greatly helpful to use the penguin remains in fecal samples to generate haplotype sequences and frequency distribution, and compare the data with fecal DNA-derived penguin haplotype diversity.