Comments to the Author  
Miller-ter Kuile and colleagues present a study on the effects of body surface sterilization on the detection of prey DNA in spiders. Surface sterilization may be desirable in molecular gut content analyses when a consumer has come into external contact with potential diet taxa; however, not much is yet definitively known about the best methods for sterilization, their effectiveness or their potential side effects.  
  
This manuscript speaks to a timely and important issue: external contamination could cause major problems for data interpretation in gut content studies. However, I worry that the results are not sufficient to address the central question of the study, and I disagree with many of the authors’ decisions regarding data collection and analysis. At a minimum I recommend extensive reanalysis of the sequence data; but better yet would be a repeat of the feeding experiment with higher sample sizes and visual ascertainment of whether the spiders actually feed.  
  
I think this project has potential to make an important contribution to the field of diet metabarcoding, but substantial work is needed before that can happen. I encourage the authors to build upon what they have done so far to make it a truly robust and reliable study.  
  
My comments on specific aspects of the study, as well as the manuscript itself, are detailed below.  
  
  
-Laboratory experiment:  
  
The purpose of this experiment was to see how sterilization affects detection of prey consumption. However, if the authors do not actually know which spiders have eaten and which ones have not (lines 149-150), then how can they reliably test for the effects of surface sterilization on detection of prey in the spiders’ guts? What is known about feeding habits of H. venatoria – do they macerate their prey, or simply suck out the digested tissues? It seems like there should be a way to tell from looking at the grasshopper remains. Otherwise, I think this experiment should be repeated and someone should observe the spiders to see if/when they feed.  
  
I am also concerned about the sample size, which is insufficient to draw reliable conclusions from the data (n=8 for sterilized, n=11 for unsterilized spiders).

*The authors agree that there are limitations to the methods used to conduct our feeding trial study. Many of these limitations are due to the remote nature of the field work and the limited amount of time available for researchers to spend at the field site to conduct multiple concurrent field and laboratory studies. At this time, we do not have funding to either return to the field site or repeat laboratory procedures (an additional funding source of $30,000+ would be needed for this combined effort), nor do we have laboratory access to conduct further molecular work due to the COVID-19 pandemic.*

*H. venatoria likely suck out digested tissue from prey items, thus, visually determining ingestion is challenging even when closely observed. We have attempted to address these two concerns in an updated portion of the discussion (lines 468-492) highlighting that these results suggest next directions and a consideration of several alternative hypotheses. Furthermore, we have chosen to switch the order in which data are presented in the manuscript, specifically presenting the field results first followed by the feeding trial results.*  
One other potential issue is that these spiders were stored in 80% ethanol without refrigeration (lines 172-173). At 80% at room temperature, the DNA of both the spider and its prey may degrade, especially when the spider is large and the water inside its body further dilutes the ethanol. The size selection method of Krehenwinkel et al. (2017) works best when the predator’s own DNA has not had a chance to degrade – e.g. when kept frozen and/or in 100% ETOH – otherwise some degraded predator DNA may be enriched along with the prey DNA. This could artificially depress the apparent prey abundance or prey detection rate in the spiders’ guts.

*We have updated this concentration based on an error we made in reporting these concentrations in the previous draft. The concentration was 95% EtOH (the highest concentration available at the field site). Furthermore, although no deep-freezing options were available at the time in the field site, we were able to keep samples stored at -20ºC until DNA extraction. We have updated these methods in lines 152-156. We agree that the use of sterilization fluids as opposed to freezing samples is worth considering, however, for potential users of diet DNA metabarcoding in similar remote field conditions, preservation fluids without freezer options may be a real constraint. We highlight that our data are promising to users in these contexts (e.g. many real-world field research projects) despite the limitation of freezer storage (lines ).*   
  
  
-Field experiment:  
  
Sample size is reasonable and experimental methods seem sound. However, I do not agree with the decision to collapse prey sequences to family level. Doing so probably obscures important variation, as a single family could represent any number of species. I therefore do not trust results addressing diversity of recovered prey DNA at such a coarse level. There could in fact be a significant difference between sterilized and unsterilized individuals that simply is not detectable at the family level.  
  
Why not forget about prey taxonomy and simply work with OTUs clustered by sequence similarity? Or even work at the ASV level (though this will probably inflate diversity estimates)?

*We have chosen to collapse prey diversity at the family level because this makes these data comparable to other studies in these types of systems (e.g. Brose et al. 2019 citation in paper). We agree that this may hide patterns at the ASV level, so we have updated our methods to examine both ASV-level and family-level richness and have found the results to be the same (lines 318-321, 420-429, Figure 3).*  
  
A bit more detail is needed on how the authors processed sequence data. How did the authors deal with NUMTs? How exactly did they determine whether an ASV matched H. venatoria or a prey item (was there a minimum BLAST % match)?

*We have included details throughout the manuscript that we hope will clarify this important point. Specifically, we assigned H. venatoria as any sequence that matched the family Sparassidae, as this is the only species in that family on the atoll (lines 109-110, lines 291-293). We have also reported more of the parameters from MEGAN highlighting the coverage of taxonomic assignments (lines 257-259, lines 389-392) to clarify how ASVs were assigned through the program. We also report the percentage rarefied reads of ASVs in each category (consumer, potential diet, non-diet lines 386-387). Specific to NUMTs, because MEGAN assigned family-level taxonomic* *assignments with 100% coverage (lines 389-392) we take this as evidence for a low representation of NUMTs in this dataset. Additionally, we consider combining taxonomic units at the family level also provides a conservative richness estimate that is less susceptible to the over-estimation of diversity that arises when NUMTs are present.*   
  
-Molecular work:  
  
The reported success rate for PCR amplification is low at 78% (lines 391-392). Did the authors attempt to optimize PCR or extractions for those that did not work the first time? PCR inhibitors can be a big problem in spiders, especially in larger individuals. It may be worth rerunning PCR using diluted DNA template, and if this does not work, then the authors could try performing a repurification of the DNA extracts.

*We optimized our PCR and extraction conditions compared to those reported in the study used to derive these results (Krehenwinkel et al. 2017). Specifically, we optimized the PCR conditions (varied number of cycles, multiple annealing temperatures, varied ratios of Ampure XP beads for all bead cleaning steps), over the period of several months as well as adjusted the concentrations of DNA template used in PCRs and ran PCRs with two different master mixes. We did re-run samples that did not work through PCR two times. We agree that this success rate seems to suggest PCR inhibitors common to spiders. We have provided our full DNA extraction and PCR amplification protocol as a supplemental document to this manuscript in an effort for these results to be fully reproduced or altered by future researchers. We have used this set of primers and this protocol (without sterilization procedure) across multiple other consumer species (n = 10, some other spiders, other insect and arachnid predators) and have found that amplification varies from 75% - 100% per species. Overall this primer set has a high amplification success rate of 97% (± 1.0% SD) across n = 262 additional samples using our optimized protocol.*

Alternatively, the MCO/Fol-degen-rev fragment may be too long for some of the most heavily digested/degraded prey DNA. The authors could try the primers from Zeale et al. 2011 (Mol Ecol Resour), which amplify a fragment of just 211 bp. Another option would be the primers of Krehenwinkel et al. 2019 (Methods in Ecol & Evol), which also amplify short fragments, but furthermore suppress amplification of spiders and therefore give much higher yields of prey DNA.

*We agree that this is a key point worth further consideration in this type of study. We chose to use the longer fragment length from Krehenwinkel et al. 2017 because it was explicitly a broader metazoan primer set with higher amplification success and which detected the most diet orders in that methods study. The consumer in our study is known to feed on insects, spiders, and vertebrates on the island (two gecko species in the genus Lepidodactylus) (line 180). Hence, we chose not to use the arthropod primers from Zeale et al. 2011 or others because of the broad diet of the consumer in this study and the evidence of success for broad diet with the chosen primer set. This method from Krehenwinkel et al. 2019 was not available when we started this study but seems very promising as a next step for this type of work. Again, because of the COVID-19 pandemic and because of the cost-prohibitive nature of the field and laboratory work to re-do these procedures, we are unable to repeat or alter these methods at this time. We attempt to address some of these important points in the current draft of the discussion and hope this is a fruitful area of future research in the field (lines 468-492).*  
  
-Writing:  
  
The manuscript generally reads smoothly and has apparently been well proofread for grammar and spelling. However, there is too much text and many sections are repetitive. Removal of repetitive text, e.g. in lines 162-173, 217-232, 298-307 and others, would probably bring the manuscript well within the word limit. Some details could be removed from the descriptions of molecular and statistical methods.

*We have removed a section of the introduction as well as several sections of the discussion that we agree were redundant (lines 80 and 446). We have replaced the discussion sections with the important caveats, findings, and next directions pointed out by your comments above (lines 468-492).*   
  
There are some serious issues with word choice that need to be corrected in future versions:  
  
“mesocosm”: This does not seem like the appropriate term for the laboratory housing of the spiders. Based on the authors’ descriptions – essentially just empty containers – I would call these “enclosures” or “containers.”

*We have chosen to change this wording to “feeding trial” throughout the manuscript and agree mesocosm is misleading in this instance.*   
  
“concatenate”: To concatenate is to string together multiple elements into a series. This is not at all the same as collapsing ASV data to the family level (e.g. line 316).

*We have changed this word to “combined”*

“taxonomy”: The authors use “taxonomy”/”taxonomies” when they mean “taxonomic level” (e.g. lines 311, 314, 317) or “taxonomic identity” (line 397).

*We agree that this is confusing and have attempted to clarify when we mean to refer to “taxonomic level” versus what we are now calling “family-level taxonomic units”.*   
  
“population consumption frequency”: This is not a well established term as far as I know. The authors should not use it in the Abstract. If they wish to use it elsewhere in the manuscript, then they should briefly define it after the first usage.

*We have removed this term.*  
  
The authors often neglect to include words like “perceived,” “apparent,” “detected,” etc. and thus imply that their experimental protocols are influencing the \*actual\* diet of the spiders rather than the results obtained from metabarcoding. Some examples are in lines 23 (“We compared diet from individuals...”) and 114-117. Future versions should be careful to make this distinction clear.

*We have included “potential” or anther such qualifier at every instance of “diet” to point out this important distinction that this DNA is perceived diet as opposed to observed.*