Associate Editor  
Comments to the Author:  
Firstly, I'm sorry for the delay in getting this decision back to you. We had a tough time finding reviewers and then unexpected availability issues with handling editors. However, we've finally got two reviews with very contrasting opinions. Reviewer 1 is advocating rejection, whereas Reviewer 2 thinks it can be accepted as is. I lean much more towards Reviewer 2's perspective, although there is some (minor) work that needs doing.  
  
In regards to Reviewer 1's comments, please take most of them as suggestions only, especially when it comes to methodology. I'd like to see your response, even if just to state you disagree. I believe they've slightly misunderstood the purpose of your study. There are some minor points here than need clarifying, but nothing more than that. Some specific comments:  
  
- I'm not confused by the abstract, this is perfectly clear. The detail they're asking for isn't appropriate for a concise abstract.  
  
- Your detail regarding Palmyra Atoll is in the right place, and you clearly state it's in the Northern Line Islands  
  
- Please disregard the yoghurt container comment, I'm not really sure how this can't be "scientifically correct". Yoghurt pots are yoghurt pots.

*Thank you for these comments and suggestions. We have attempted to address the clarifying comments of the reviewer and respond with no changes where we deemed the reviewer misunderstood the purpose of the study. We hope that these terminology and methods clarifications help with making the manuscript clearer.*   
  
Reviewer(s)' Comments to Author:  
  
Reviewer: 1  
  
Comments to the Author  
In general, the abstract is confusing. Several methodological aspects need to be clarified. It is not clear enough how many spiders were collected, how many of them were analyzed with and without sterilization treatment (I don't know if this is the most appropriate term) and how many were taken to the laboratory for feeding. In the bastract, it should be included how many otus were identified in the spider's diet.

*We have attempted to aid in clarity throughout (primarily addressed in the line-by-line comments below). Thank you for your time in reading our manuscript and we hope that our edits have aided in its clarity.*   
  
In the line 26, the auotors mention: natural environment. indicate where they were collected.  
In line 27, the auotors mention that ``using DNA metabarcoding of full body parts (opisthosomas). the authors should clarify whether they used the whole body (opisthosoma + prosoma) or only the opisthosoma.  
In line 27 to 28, the authors say: We compared diet from individuals surface sterilized. what do they mean by sterilized individuals? were the spiders or the preys or surfasse sterilized? please clarify  
In Line 30. Same comment above

*Thank you for these comments in the abstract. Because we were aiming for a concise abstract format, we have omitted some of the details you request here and include them later in the introduction to the field site as well as the methods used to sterilize the individuals. Because the purpose of this study was not ecological, but rather methodological, the important aspect of the consumers collected in their natural environment is that we were examining the influence of surface bleach sterilization on what is detected as “naturally occurring diet”, and thus do not need to provide further ecological details here. We believe that specifying that the body part we used was the oposthosoma is clear enough here – we do not include other terminology (e.g. prosoma) because of the brevity asked for in the abstract and because we believe adding more terms will confuse as opposed to clarify. We have added the word “consumer” prior to “individuals surface sterilized…” in line 29 to increase clarity that we surface sterilized the consumers as opposed to prey/diet items. We have also re-arranged the words in the title of this article to reflect a similar clarification of what was surface sterilized. Furthermore, we specify that the sterilization treatment was aimed at removing contaminants (line 29) as per requests for clarity.*

In line 73 to 74 , the authors say: However, surface sterilization has not been systematically used in diet metabarcoding studies. This sentence is contradictory to what was mentioned above. see lines 71 to 73. For example, Liu et al., 2020 recommend standardized sample cleaning prior to DNA metabarcoding analysis but not sterilization as such. This treatment could affect the DNA extraction process. Washes with ethanol or distilled water solutions are usually used. Liu, M., Clarke, L. J., Baker, S. C., Jordan, G. J., & Burridge, C. P. (2020). A practical guide to DNA metabarcoding for entomological ecologists. Ecological entomology, 45(3), 373-385. Furthermore, the word sterilized could be replaced by cleaning or elimination of organisms such as small insects, etc. if sterilization is sought, it refers more to the disinfection of microorganisms. is this what is sought?

*We agree that it is important to consider results within the context of previous work, so we appreciate your comment here. The previous sentence highlights that surface sterilization of samples themselves is used in some fields that utilize DNA metabarcoding, though, importantly, this paragraph highlights that though the practice is common for some fields (e.g. fungal endophyte research), it is not used systematically in diet DNA metabarcoding. The paper you suggested is a wonderful resource for determining species composition and richness from eDNA samples, but there is no specific guideline in that paper for diet DNA metabarcoding, in which the aim is to determine the DNA composition of prey* ***inside*** *a consumer as opposed to the composition of a bulk sample from an insect trap, for example. Furthermore, that paper highlights the importance of surface sterilizing equipment as opposed to samples themselves, and we have made many efforts in this study to ensure the sterile nature of equipment used in the field and laboratory settings. We have aimed to increase clarity that we surface sterilized the consumer themselves by re-orienting the title of the article, and we hope this makes it clearer that this study is about the surface sterilization of consumer samples as opposed to surface sterilization of equipment.*  
  
In Line 89, the sentence, full body parts (opisthosomas) should be clarified. See previous comments.

*We have clarified that this was without internal dissection in now line 91.*  
  
The paragraph from lines 88 to 101 should go in materials and methods; not in introduction.

*While this paragraph has some methodological details, we feel that it is a key part of the introduction to the study we performed, which is a methods-validation study. The authors included enough detail here to inform a reader of the intent of our study and its relevance in the field. In placing this paragraph here, we have followed guidelines suggested across ecological fields in the format of scientific writing and the important details needed in the introduction for readers to understand what we did in our study and why (we follow recommendations from this blog post: https://dynamicecology.wordpress.com/2016/02/24/the-5-pivotal-paragraphs-in-a-paper/)*  
  
In line 105, the country must be indicated. at what time were the spiders collected? If the species (I don't know) has nocturnal home habits (like many spiders that do not produce webbing), this data would be crucial to interpret the results.

*We have added the country (USA) at now line 108*  
  
In lines 106 to 108, the sentence, Palmyra Atoll has a well-characterized species…..does not belong to materials and methods.  
In lines 115 to 119, in Natural environment consumer collection,  how many females and males were collected? Were all spiders collected as adults? In spiders in general, sex and age are determining factors in trophic ecology, so they must be taken into account.

*Clarifying the species list of Palmyra Atoll we believe is an important ecological context and justification for our study – showing that this is not a very diverse system so quantifying it is easier than a more diverse system. Again, we have followed the writing norms in the ecological field to give ecological context and justification for the study system we chose for this study.*  
  
In line 123, delete or replace the phrase one-liter yogurt containers with holes…is not scientifically correct, even if it was done in reality.

*We have kept the explanation as a yogurt container because in the ecological field these are commonly used in these contexts. However, we have specified that the yogurt container was plastic (Line 131).*  
  
The experiment in Feeding trial consumer set-up and feedding; lines 121 to 128, not sufficiently supported. why do DNA metabarcoding analysis on spiders with mono-diet (one large grasshopper) treatment? is it supposed to be a controlled environment with less chance of finding contaminants?

*The laboratory trials were included to provide a controlled environment in which we knew what diet items the consumers would be consuming. This was a way to validate that we detect diet when we expect to find it. Furthermore, because these feeding trial environments are commonly used in trophic ecology – this was a way to explore the risk of surface contamination in this more contained setting common in the field. We have not changed anything in this section and hope that our explanation helps contextualize this part of the study better.*

 In Line 143, What do you mean by hood and the opisthosoma (hind gut region)? is this opisthosoma + prosoma region?

*The opisthosoma is the hind gut region of these spider predators (explorations in Krehenwinkel et al. 2017 and Macias-Hernadez et al. 2018 on use of this body part in spider diet metabarcoding studies). We specify both here for non-spider ecologists and spider ecologists alike to understand the type of consumer body part material used. The laminar flow hood is a piece of laboratory equipment designed to maintain sterile conditions for samples while working with them in the lab. WE have attempted to restructure this sentence in lines 151-153 to aid in clarity of these points*

In PCR amplification (Lines 161 to 169), authors should considered the following aspects: Justify the non-use of blocking primers; widely used in dietary analysis with spider DNA metabarcoding.

*We agree that blocking primers are a useful tool in diet DNA metabarcoding studies. We chose not to use them because our aim was to provide a general protocol that could be used for many different kinds of invertebrate diet DNA metabarcoding studies, not just the species specified here. Because we hope that ecologists who may want to build off this work to examine food web level patterns (e.g. multiple species of consumers), we wanted to test our methods without the use of species-specific blocking primers, which would be time prohibitive when scaling diet DNA metabarcoding up to a consumer community versus one consumer species. We agree that for studies of specific species, blocking primers are a very useful tool for increasing diet DNA, though we follow methods from others in the field (e.g. Krehenwinkel et al. 2017) that demonstrate these blocking primers are not necessary to extract diet DNA.*  
  
In line 185, does the term amplicon sequence variants (ASVs) refer to OTUs? what is the difference?

*There is a difference between these two terms and so we use ASV throughout to specify that these are the sequence variants we have used. The methods used to cluster around these two methods vary – whereas OTUs represent some cluster of sequences that are similar (say, at 97% similarity), ASVs use exact sequences, their abundances, and error models for a sequencing run to give confidence to each sequence and whether it is due to sequencing error or, regardless of relative rarity, likely represents a real sequence as opposed to sequencing error. There are many resources explaining the distinction, but here is one from Zymo Research: https://www.zymoresearch.com/blogs/blog/microbiome-informatics-otu-vs-asv*  
  
In lines 229 to 239 Filters must be applied to eliminate OTUs that do not correspond to potential spider diets. For example, bacteria and fungi that are normal inhabitants of the intestinal flora of insects. this explains why the results show OTUs belonging to the intestinal flora. These filters should be included in materials and methods, explaining how the filters were made. What is the level of taxonomic indentification?

*Our potential diet item filter included all ASVs in the Kingdom Animalia and the Clade Bilateria (line 242-243), since this includes all invertebrate and vertebrate organisms on Palmyra Atoll. We have specified this filter in line 244. We have specified in the results which DNA corresponded to non-diet items (Lines 301-302). We considered anything that was not fungi or bacteria to be diet items – as this is a generalist consumer and the species pool of the field site is relatively species poor and only includes primarily other invertebrate arthropods that are all likely prey along with one set of vertebrates (geckos) that are also observed prey items. (Justified more in the methods paragraph from lines 107-117.)*   
  
Results  
In lines 284 to 290, the OTUS of spiders collected in the wild and those on monodiet treatment are mixed. They are completely separate events. it is not clear why.

*We do not fully understand this comment. However, because these samples compose the same species from the same field site and were also sequenced on the same sequencing run, we performed the UNOISE3 and DADA2 cleaning, filtering, and denoising algorithm on all samples in conjunction. Only after that did we separate the two sets of samples (natural and feeding trial) due to differing sample preservation treatments and sample ages – these both can influence DNA extraction and sequencing outcomes.*  
  
Same observation in Lines 302 to 304

*We agree that the lack of detection in some consumers from the feeding trial may have several causes. One ecological one is what you have pointed out – that these consumers did not, in fact, ingest the diet item while in the feeding trial. We have expanded on this result as a next direction in the discussion (Lines 384-398). Another cause could be degradation due to the bleach wash, however, other studies of soft-bodied invertebrates in which bleach washes have been used did not seem to degrade DNA to the point of non-detection, so we feel confident that this is not the only reason for this lack of detection.*

In line 286, say.. 56 of 72 initially extracted samples successfully amplified and sequenced (Table 1)… Depending on the time the spiders were collected, it is possible that several of them did not consume potential prey, but it is not understood how in the treatments in which prey were supplied in a controlled manner they did not amplify 100%.  Is it possible that this is an effect of not having used blocking primers? any other explanation?

*This is an important point that we appreciate you have brought up. We have aimed to clarify that only successfully amplified samples were sequenced (line 298-299 and in Table 1). The samples that did not successfully get amplified genes following PCRs were not sequenced. Because we detected consumer DNA in all samples, it is unlikely that this amplification failure was due to a lack of diet DNA. Rather, it is likely that, common to other studies of spiders, that there are PCR inhibitors in these samples. Because we got relatively high success during amplification and because our aim was to create a scalable protocol that could apply across many different invertebrate consumers, we did not optimize the removal of these PCR inhibitors in this study.*   
  
In lines 314 to 330, the results should be expanded, presenting the proportions of the taxonomic groups as the basis of the spider diet; as well as the differences in items per individual. Prey potential is poorly described

*We expand on the prey composition in the following section (Lines 334-345) and in figure 4, which shows the presence and abundance of prey items across individuals from both treatment groups. We also have a supplementary figure (Supplementary Figure 8) which shows the abundance and presence of each diet sequence across all individuals. Because the individual-level analyses and the trophic ecology of this spider species are not the primary purpose of this study, we do not include that figure nor the taxonomic composition of those diet items in the main text, but they can be observed in both the y-axis of Figure 4 as well as in the supplementary figures 6-8. We have added a section on the most common diet orders in lines 342-345).*  
  
In the discussion, in lines 333 to 382, the authors focus on discussing the effect of sterilization and, as a consequence, contamination on DNA metabarcoding analysis. Although the authors show that there are no significant differences between the disinfected (a term that should be changed to decontaminated), the discussion of this topic does not provide support for the true contribution and impact of these results in future DNA metabarcoding dietary analyses.

*We are not sure what specifically is missing from our justification and how it applies to future studies. We wanted to avoid being overly confident and prescriptive of our results and their implications but suggest in lines 359 – 360 that it does not appear that surface contaminants, and thus, surface sterilizing, seems necessary. However, we wanted to provide caveats and next directions to this finding in following paragraphs by suggesting contexts and steps that might illuminate more nuance (lines 384-398). Furthermore, we have chosen to maintain our terminology of surface sterilization, but specify that it is to remove surface contaminants, in various parts of the manuscript (e.g. lines 29, 88, 95)*  
  
although Diet DNA metabarcoding is being discussed (lines 384 to 397), there is no discussion on the central issue of DNA metabarcoding analysis in this spider: what is the food base of H. venatoria? What taxonomic groups or species are common or different from those observed in other spiders? What is the contribution to this topic? there is nothing!

*While we agree that these are all interesting points that we hope to explore in our work, and we hope the field continues to build on, because this study is a methods-validation study, we do not feel that many of these details are necessary additions.*  
  
In line 617, What is the title of the figure? In a) it is not clear how the 100% prey detection rate was obtained, if the results and table 1 indicate the opposite. it is not clear

*We have addressed many of these comments in the text (e.g. Lines 384-398). We do not believe that it is the practice of the journal to require figure titles, and so we do not provide them. As mentioned in the text, any samples that were not successfully amplified were not sequenced (line 298-299 and in Table 1), and so 100% detection rate represents just the detection in sequenced samples. The lack of detection in the surface sterilized individuals, as discussed in the text (lines 384-398), may be due to the fact that ingestion was not confirmed or other causes, which is a main point of our caveats/next directions.*  
  
In figure 1, section b) it is not clear why we did not obtain 100% detection of the prey on the stellitized surface. Could it be the effect of the disinfecting agent as an inhibitor of PCR?

*We have attempted to address this concern above comment and caveat text in discussion lines 384-398.*  
  
In line 661, What is the title of the figure? Also, the results in figure 4 are neither expanded in results, nor discussed!

*Again, we do not believe that figure titles are necessary for this journal. Furthermore, this figure is contextualized in both the methods section (lines 269-277) and in the results (lines 335-345) and represents diet composition of consumers from natural environments that were and were not surface sterilized.*  
  
Reviewer: 2  
  
Comments to the Author  
This is a really nice, small but timely and important study that addressed the important question whether to sterilise or not sterilise specimens prior to proceeding with gut content analyses using DNA metabarcoding. Opinions on this topic differ immensely and thus it is fantastic to see a study that just makes the important test with a sufficient samples size. To me all methods and results are clear and the conclusions derived perfectly fine. One could consider condensing the manuscript a bit but as this is an online journal I would just leave it as is to have the carefully written methods details at one place.

*Thank you for your comments on our manuscript.*