Review - Miller-ter Kuile et al Miller-ter Kuile and others have conducted a worthwhile study on the impact of surface sterilization on prey item detection in spiders from the natural environment as well as from mesocosms. They have done an excellent job providing detailed, well-documented laboratory and analytical methods. The supplementary material is a great asset to the paper.

There are two main issues with the manuscript. First, I found the written section about the sterilization procedure(s) confusing. Perhaps I have misinterpreted, but it seems as though bleach is only used as a sterilization method for the spiders from the mesocosm experiment. I would recommend re-writing that section to clarify which sterilization methods were used for which spiders.

Most importantly, the biggest issue concerns the interpretation of the mesocosm results. There is no such thing as “marginally significant,” and suggesting that the results are marginally significant (p = 0.07) is a misinterpretation of the results. A substantial re-write will be required to address this misinterpretation in the abstract, results, and the discussion. I have discussed this issue in more detail below (under lines 409-410)

Please find more detailed comments below:

Abstract lines 23-25 Were the spiders from natural environments sterilized in bleach or via a laminar hood (presumably via UV)? This needs to be clarified in the methods and, if necessary, clarified in the abstract.

Lines 27-28 There is no such thing as “marginally significant” (p = 0.07). A more detailed discussion of this point follows below (lines 409-410).

Introduction line 52 “form the base of most food webs” Please clarify \*terrestrial\* food webs

Corrected

line 66 Please provide citations.

Provided citations

Lines 93-101 This argument about the potential of contamination to “inflate” or “depress” estimates of consumption is a bit confusing, For instance, if you are considering alpha diversity, contaminants that are not potential diet actually inflate estimates of consumption. Please state in other terms for clarity.

Removed entire section for clarity in the introduction given that we discuss this in the discussion (FIND LINES)

Line 104 To clarify beyond “used in analyses” perhaps it would be better to write “used for diet DNA metabarcoding”

changed

Lines 104-105 It is awkward to state “high throughput sequencing results of the COI gene…” Rather, you could state “Targeting the COI gene region, we produced high throughput sequencing results….” or “We performed COI DNA metabarcoding with the full….”

Changed

Line 107 No comma necessary

Changed

Line 114 By abundance, do you mean relative abundance?

Lines 115-118 To reduce verbosity and over-interpretation in the intro, remove “suggesting that contaminants either hide or inflate diet consumption amount” and “suggesting that surface contamination could alter ecological interpretations of community-scale species interactions”

Changed

Line 130 For the non-arachnologists, what kind of prey do these spiders typically target? Based on the Handler et al (2007) reference, do they mostly consume arthropods? It is probably worth explicitly mentioning what they typically ingest.

Line 131-134 It seems out of order to explain the collections in 2017 before the collections in 2015.

Lines 142-143 According to Macias-Hernandez (Figure S1), in the opithosoma of the woodlouse hunter spider, woodlice could be detected for over 100 hours after feeding. At 50 hours after feeding, there was a nearly 100% positive detection rate. In comparison, waiting only 12 hours in the present study to allow for the digestion of previously consumed prey items seems like a very short time period. What is the reasoning behind waiting only 12 hours in light of the cited reference?

Lines 169-171 2 minutes is a very long time to submerge an small-bodied organism in 10% bleach. It is my understanding that surface sterilization with bleach is generally effective within a much shorter time period. I wonder if the bleach can seep into the exoskeleton of the spider and damage the internal DNA after 2 minutes? Can you speak to the permeability of an exoskeleton?

Lines 155-182 The whole surface sterilization section is confusing to follow – it would be helpful to clarify when and what was done to each set of samples (natural vs mesocosm). Were the natural environment consumers sterilized in bleach before being frozen at -80C? It sounds like they were immediately frozen (lines 157-159), then they were sterilized in a sterilized laminar flow hood before extraction? If I am not misinterpreting, then it sounds like only the mesocosm spiders were surface sterilized in bleach, while the natural spiders were sterilized in a laminar flow (UV)? Using different methods of sterilization could pose significant problems for comparability, although this isn’t necessarily the point of the study, so it isn’t a fatal flaw. From the abstract, I was under the impression that bleach surface sterilization was the only method of sterilization used in this study. I’m hoping this is a misinterpretation on my part…

Line 189 “….lower molecular weight consumer or diet DNA…” Is the “or” a typo?

Rephrased to “to isolate a proportion of lower molecular weight DNA prior to PCR steps…” to increase clarity.

Lines 190 “DNA prior to PCR steps with Ampure XP beads” It sounds like Ampure XP beads are part of the PCR steps. For clarity, please re-word as “…DNA with Ampure XP beads prior to PCR”

Changed

PCR amplification, library preparation, and sequencing + bioinformatics sections Great - nicely detailed, easy to read.

Lines 291-294 (similar issue to lines 93-101) This concept is confusing: “…increasing detection because of “false” diet detection or by decreasing detection because of abundance of non-diet DNA…” Although I understand the authors’ intentions, this could be misleading since you could equally say that including non-diet DNA items equals increased detection, depending on how “detection” is defined. This can either be reworded or “detection” can be more clearly defined.

Line 311 Should use “that” instead of “which”

Changed

Line 317 Should use “that” instead of “which”

Changed

Lines 319-323 The GLM descriptions were incomplete here, but then I saw that much more detail is provided later in the methods. I would recommend putting all the GLM information in the same section (probably the latter section) to prevent confusion.

Lines 327-330 “This is especially important…” This does not belong in the methods. This could be relevant information to include in the intro. However, it’s unclear what the other ~92% of DNA represents in that study: host tissue, likely contamination, or other? I would think that host tissue, not contamination, would be responsible for swamping a sequencing run to that extent, which would make this argument obsolete in the context of this paper.

Lines 397-399 Perhaps I have overlooked the definition of “potential diet items,” but if 23% of the taxonomicallyassigned ASVs correspond to potential diet items and 8% of them correspond to consumer DNA, what do the remaining 69% represent? Could there possibly be more diet items unknown to this spider before this study? Or is all of this DNA considered secondary predation (prey items of the prey items)?

Lines 409-410 There is no such thing as “marginally significant.” If you choose to use frequentist stats, which is perfectly acceptable, then you must also accept the significance level of 0.05. Frequentist statistics is based on the idea that the null hypothesis will be rejected 5% of the time even if it’s true. Otherwise, you are interpreting your results with post-hoc bias. Naming p-values between 0.06 and 0.10 as “marginally significant” is a misinterpretation of the data, and it needs to be corrected throughout the manuscript. This includes the language surrounding the results as well as the interpretation of the data in the discussion and abstract. It’s okay to have non-significant results. I also wonder whether the long bleaching period led to the decreased detection of offered prey items. How permeable is the exoskeleton of a spider?

Line 422 Should use “that” instead of “which”

changed

Lines 438-447 This is a misinterpretation of the results. This section needs to be re-written to account for the results: surface sterilization before metabarcoding does not change our perception of diet in natural environments or mesocosms. It may also be worthwhile mentioning that two different sterilization techniques were used (if that is indeed the case), so it is difficult to directly compare the natural vs mesocosm results.

Lines 471-474 This needs to be tweaked. It is okay to discuss why mesocosms may be more prone to contamination; however, the language should not say that the study provides “some evidence” that mesocosms are more prone to contamination.

Line 489 Should be “further” not “farther” because it is an expression of time, not distance

Corrected

Lines 500-509 This recommendation cannot be made in light of non-significant results.

Supplementary Information – Thank you for providing such thorough, detailed information.