**Shaena Montanari’s Response to Reviewers: Resubmission #2**

Reviewer: Roberto Ambrosini

**Basic reporting**

No Comments

**Experimental design**

No Comments

**Validity of the findings**

I found this new version of the paper improved and much easier to read than the previous one. The author also modified the text according to most of my comments. Unfortunately, there is still one important methodological aspects of meta-analysis that was totally overlooked during the revision. I am referring in particular to my previous comment on how the author accounted for non-independence of data in the meta-analyses (“Did you account for non-independence of data reported in the same paper or by the same research group in the analysis?”). I found no specific answer to this point. The author stated that regression analysis and ANOVA was used, but these analyses did not account for non-independence of data, which is a major issue in meta-analyses, because results from the same study are less independent to one another than those from different studies (indeed, they share the same methods, the same people making the lab and the statistical analyses etc.). The results of meta-analyses can be seriously biased if non-independence of data is not accounted for.

There is also a second important issue with meta-analyses, which I missed in my previous comments. The author apparently did not account for the variance of the results from different study. For instance, in this study, delta-15N is estimated to be 4.6±1.8‰ (by the way, the author should state whether 1.8 is the standard deviation or the standard error), but then only the value 4.6 is used in the meta-analysis. I guess that all the other studies reported standard error/deviation for delta-N and delta-C values, but they were omitted from the meta-analysis. This is a large and unnecessary loss of information because meta-analytic procedures allow accounting for variance in the results.

As far as I can see from the tables in the manuscript, the meta analyses should be conducted by using mixed models, with study as a random grouping factor and diet or body size as a random covariate within study. Each delta value should also be weighed by the reverse of its variance. However, the author should consider these my advices as a very rough guidance, as more problems might be hidden in the data. For example, data from the same species are non-independent. However from the tables it appears that each species was investigated in one study only, so in this case study should account for both variability among studies and among species. Phylogenesis might/should also be considered. I therefore strongly suggest discussing in details the analyses a statistician with an expertise in meta analyses. I can also suggest the excellent paper by Van den Noortgate et al (Three-level meta-analysis of dependent effect sizes. Behav Res 45:576–594, 2013) for methodological details on meta-analyses.

**I have spent a few months thinking about these comments and asking other statisticians and come to the conclusion that the available data are not sufficient for a meta-analysis for the following reasons. My main goal was to see if there is any relationship between the trophic discrimination factor (TDF) values and body size or dietary isotope value. To do this, I need to combine data from many studies. The issue I’ve realize when reassessing the data is that most of the TDFs from these studies are not significantly different from zero or do not provide enough data to determine if they are, meaning comparing them across multiple studies will provide no useful information. There is no use in looking for trends in values that are not statistically significant from zero.**

**I would still like to present the TDF values in tables to show the body of work that has been done on the subject, but they can only be talked about in qualitative terms. Other papers I have cited doing ANOVAs of TDFs from a variety of studies are inaccurate because they don’t take any of this into account and this mistake should not be repeated because the numbers being compared are arbitrary if they are not significant.**

**I greatly appreciate the time you made in making this comment because it helped me reexamine the data and realize I did not have enough power in my dataset to do it and produce valuable results.**

**Comments for the author**

Minor comments
Line 45. I would add a short statement on why small mammals are often overlooked. I guess because they are often elusive.

**Elusive and also overlooked in favor of larger animals. I’ve updated this.**

Line 49. It is a bit unclear what the author exactly means with "baselines"? General conditions?

**I changed baselines to conditions.**

Line 81. I would add here, rather than below, that the discrimination factor is also called trophic discrimination factor. I think that adding this further definition later adds confusion.

**I clarified these definitions.**

Line 161. How did you graphically examine data to assess normality of distribution? Did you use qq-plots? Please specify. I would also suggest using Levene’s test instead of F-test to assess equality of variance, because the F-test is known to be extremely sensitive to non-normality.

**I put a Shapiro-Wilk test in the text.**

Line 207 and elsewhere. Please, report degrees of freedom for all F statistics.

**Added in the text.**

Line 211 and elsewhere. Please, report also the residuals degrees of freedom for all F statistics.

**I have removed the least-square analysis so this is not needed.**

Table 4. In the table (not in the legend) please use t-test and Welch t-test instead of t-test equal and t-test unequal.

**Clarified in the table.**

**Reviewer 2**

**Basic reporting**

Figure and table legends should be improved so that they contain all necessary information.

**Experimental design**

No comments.

**Validity of the findings**

I’m not very happy about how the main result of the experiment is presented. I think there has been effort to present it as more general as it is. Although some comments have been added concerning daily variability in the feces isotopes the fact that the discrimination value that is calculated is an average value for feces of omnivorous animals using an average of food items (and not necessarily the food items they had consumed the day before) and not necessarily the correct discrimination value for any random feces of that animal is not admitted. This would be important if someone wants to track changes in diet in short scales.

**I apologize for it coming across this way and I tried to not make it sound too general, but I have added further comments noting these points and hope it is clear I am not presenting it as general but rather one case study.**

**Comments for the author**

Title: I would add something that shows that there is some literature review as well.

**I have kept the title the same because I changed the lit review a bit.**

Line 33: trophic discrimination of what?

**I have clarified this to say “diet-to-feces”**

Also in the brackets there should be information that can be missed without impact on the content, here you don’t explicitly say it’s carbon and then later there is a Δ15N while you haven’t said that Δ is trophic discrimination factor but it seems as ‘Δ13C’ is trophic discrimination carbon.

**I have clarified this section and hope it is clear.**

Line 37: ‘more directly’ compared to what?

**I removed this phrase.**

Line 39: ‘A meta-analysis shows that unlike discrimination factors from other tissues and materials’: Rephrase. Your meta-analysis did not deal with other tissues so I wouldn’t start the sentence with ‘a meta-analysis shows…..’ because it could be confusing and someone might think that your metanalysis was bigger that it actually was. Also which other materials you refer to here? Normally the samples can be tissues or feces.

**I have removed the talk about the meta-analysis.**

Line 40: in which isotopes?

**Inserted “carbon and nitrogen”**

For example Lines 52-53 and 59-60: citations can be in random order? Not alphabetically or chronologically?

**I’ve put them all in chronological order now.**

Line 82: why don’t you give here all synonyms of trophic discrimination and the TDF?

**I have listed additional synonyms.**

Line 85 for example: feces is not a tissue and here it’s talking about tissues (so not for feces). In these cases I would suggest the use of the word ‘sample’

**I have clarified mentions of ‘tissue’ in relation to feces and put ‘sample’ or ‘byproduct’.**

Lines 167 and on: how did you compare between lipid and non-lipid extracted samples? It’s not clearly said.

**In the results in line 204 I explain I compared them with a t-test.**

Line 169: ‘results’: which results exactly?

**I have clarified this as “stable isotope results”.**

Line 204: did you compare (with a statistical test) between the 4 weeks?

**I had not done this, but I just did and there is no statistically significant difference between weeks. I have added this analysis to the paper (Line 240).**

**The following four comments are no longer needed since I changed the meta-analysis in this paper:**

*Line 284: ‘it appears no such relationship with feces exist’ you just said that in before in the previous sentence*

*Line 301: Did you do this test also between groups or for each group separately? (omnivores, carnivores and insectivores)

Lines 317-318: Does this variability have to do with the size or the diet? Since they are omnivores, a variability should be expected no matter of the size I would say.

Line 330-331: here you always talk about the average of feces and the average of the diet. I find not precise your statement. If you had taken one feces produced from the real food item that was consumed the day before you don’t know what the difference would be.*
Line 333-334: ‘or by the addition of microorganisms in the gut during gastrointestinal transit’: how did you results show this? To me it seems unsolicited comment following the ‘also show’

**This was conjecture from another paper but I am removing ‘microorganisms’ because I did not cover it sufficiently in my experiment.**

Delta that is δ is not explained anywhere but suddenly ‘delta’ is used

**I have removed the word “delta” and replaced it with ‘isotope ratios’.**

Line 150: all feces and diet samples were subsampled? I find this unnecessary. You could put the effort, time and money instead to analyse more feces.

**Not all, just some to test the effects of lipid extraction. I could only get as many feces as the zoo was willing to collect for me.**

Line 219: ‘body mass’ redundant, if you say 5-100kg should be enough

**I’ve removed this.**

Lines 229-239: it is not clear when you talk about nitrogen and when about carbon. There doesn’t seem to be an order. E.g. line 229 which isotope?

**I have clarified which isotope I’m talking about.**

Line 237: this is in mammals, not general

**I have emphasized “mammalian”**

Line 241: it looks like you repeat something you said in the previous paragraph

**I have clarified redundant language.**

Line 243: it is Salvarina et al. (2013) not Salvarina (2013)

**I added et al.**

Figure 1: a fullstop is missing after the word ‘sampling’. You could mention that it is feces of meerkat.

The legends in general are not informative enough. They should stand alone and provide all information needed to understand what they show. Please also mention the word ‘meerkat’ and feces whenever needed.

Table 4: if someone reads only this table legend has no idea what it is about.

**Overall I have tried to make the figure and table legends more descriptive.**

Why do you use sometimes scat and sometimes feces?

**I have updated all mentioned to “feces”**

Line 575: Doesn’t the scientific name need to be in italics?

 **I have gone through and fixed all of these names in the references.**

Table 1: your reply to my previous comment was ‘Perhaps I am misunderstanding but Table 1 in Salvarina et al. shows the ‘light labeled’ diet Δ13C for R. ferrumequinum to be negative. That is where I obtained this value.’

Please check again table 1 and figure. δ13C for R. ferrumequinum feces is the same as the diet (light labeled diet).

**I have updated this entry to be 0 for Δ13C.**