Stacks

Peer Review: Parts of a whole: isotopic difference between single keratin-based tissues and whole-body tissues of birds and mammals

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Accepted by 2 of 2 reviewers

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The authors declare no conflicts of interest.

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Transparent Peer Review

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- View resubmission with reviewer comments and author responses
- View initial submission with reviewer comments and author responses

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Reviewer Summaries

Christopher Brodie

Initial Submission

Do you have any conflicts of interest that could bias your ability to provide an independent review?

No

What did the authors do a good job with?

Challenging widely held assumptions in the field and designing a study to obtain measured data and interrogating those assumptions. The authors should be applauded for taking on such a fundamental study that can help support confidence in data interpretation and guide future studies.

How do you think this research will contribute to the field?

It draws attention to fundamental assumptions, which are often just that, an assumption. The consequence is it provides a new line of through for future studies that will benefit everyone, and the wider field. It calls for ensuring the first principles are right, before getting into the stories.

Regarding the study design and methods, what do the authors need to fix or improve upon to be fit for publication?

The authors must add:

(i) Details of the materials they used in their analytical sequence to normalize their measured isotope values from their samples to the VPDB and AIR scales for carbon and nitrogen respectively.

(ii) Calculate and include measurement uncertainty and state exactly how they calculate measurement uncertainty on their samples, before undertaking any form of data evaluation procedures.

(iii) An explanation of how the mixing models work and are used, how the models are using mean value data and incorporating measurement uncertainty into their summaries.

(iv) An explanation on why p-values are used and why they are valid for interpretation and why using threshold differences in measured stable isotope values that have been normalized are not valid.

(v) Contextualize the conclusions in context of published data in the specific research field.

(vi) Explicitly explain the “mathematical correction”, how it should be used, at what point, and lay out what assumptions are being made in using it – key to this is being explicit, using evidence, of what the mechanism is for each sample type and that it is reproducible.

a. Any shortcomings must be explained with guidance on how to overcome them (e.g., does each study need to assess this in a small way?)

(vii) Be more thorough in next steps / future work / minimum required work in any future studies to ensure a sound approach to dietary explorations.

Regarding the analysis and interpretation of their findings, what do the authors need to fix or improve upon to be fit for publication?

Measured stable isotope values must be presented in the context of its measurement uncertainty, and then if interrogated (e.g., using statistics or mixing models), done so in the context of that uncertainty. I strongly encourage the authors to then consider how the measurement uncertainty envelope expands once such data interrogation methods are employed, and how they capture that added uncertainty and include it in their results summary before interpreting the data in the context of the research objectives.

To be able to present measured stable isotope values in the context of a discussion on correcting the data relative to a specific, reproducible mechanism that has caused isotopic fractionation during animal lifetime, and especially when they are being proposed as unidirectional linear corrections, the proposed correction must be presented as being robust.

In the current manuscript version, the authors make a statement at the end that calls for the application of a linear offset of data (-1.97‰) and provide an uncertainty of ±0.37‰ on that value. However, it is not clear what the rationale for that is, where this uncertainty came from and what it represents in the context – is the ±0.37‰ just a model estimation envelope and, if so, where did it come from and how representative is it?

Moreover, the authors should comment on what the uncertainty on that proposed offset means for other studies, and how other researchers should interpret it. For example, 1.97±0.37‰ is -1.60‰ to -2.34‰, so what data should be used, and how should that data be used by others? The authors need to offer guidance and state the assumptions and limitations of proposing linear offsets on data and how sound they are.

It is also important to have a clear and explicit explanation on why statistics are used and why they are adding to the interpretation strength of the findings – why are p-values leaned on so heavily in this manuscript? The same goes for how the mixing models work and how they model interpretative uncertainty (i.e., measurement uncertainty plus the uncertainty added by modeling / statistics).

Is there anything else you think the authors need to fix in their article to be fit for publication?

I have added comments throughout the manuscript. The authors should improve:

(i) The section on how measured stable isotope values have been calculated, corrected, and normalized

(ii) Measurement uncertainty should be calculated and presented on all data

(iii) Fully explain and justify the use of statistics in the context of the study and published literature

(iv) Elaborate on the mathematical correction they propose, clearly showing the mechanism has been identified, that it is reproducible, and that the proposed offsets the authors state is sound and valid. This is not clear in the current manuscript and, ultimately, does not have a reasonable uncertainty envelope on the data, therefore, in my opinion, cannot be consider reasonable or valid in it's current form. Nonetheless, the authors ought to have the data and information to hand to re-evaluate this and include it in their manuscript without further analysis.

a. This does require an understanding of how the analytical sequences were setup to determine, however.

Do you have any concerns about the ethics of this research?

None, on the provision that the sourcing of the animals via the cited company can be proven to meet the appropriate ethical standards for the research.

Do you believe the article, in its current form, is fit for publication?

Revise & Resubmit

Revised Submission

Do you have any conflicts of interest that could bias your ability to provide an independent review?

NO

How well did the authors respond to your comments?
2/5
What - if any - feedback do you feel the authors did not adequately respond to?
Measurement Uncertainty.
Based on your review, what should happen next?
This paper needs major revisions and another round of peer review
Based on your review, what should happen next?
This paper requires minor revisions but does not need further peer review
Would you like to be listed as a Collaborator on the final publication?
No, I do not want to be listed as a Collaborator

2nd Revised Submission
Do you have any conflicts of interest that could bias your ability to provide an independent review?
No
How well did the authors respond to your comments?
2/5
What - if any - feedback do you feel the authors did not adequately respond to?
Measurement Uncertainty and assessing their data offsets in context of that and their precision
Based on your review, what should happen next?
This paper requires minor revisions but does not need further peer review
Would you like to be listed as a Collaborator on the final publication?
Yes, please list me as a Collaborator

Ashlee Mikkelsen

Initial Submission
Do you have any conflicts of interest that could bias your ability to provide an independent review?
No
What did the authors do a good job with?
the authors did well at identifying a crucial gap in our understanding of diet, foraging ecology, and using stable isotopes to estimate diet
How do you think this research will contribute to the field?
I think the research highlights several issues in stable isotope analyses that we as practitioners often ignore and they attempt to provide a solution
Regarding the study design and methods, what do the authors need to fix or improve upon to be fit for publication?
The authors need to invest more time in carefully describing model selection criteria and their hypotheses their models are testing.
Regarding the analysis and interpretation of their findings, what do the authors need to fix or improve upon to be fit for publication?

The authors make a pretty big promise in the introduction when they identify the knowledge gap, but I don't feel like they follow through on the promise. I would like to see a specific proposed solution to the problem they identify and clear benefit to their solution

Is there anything else you think the authors need to fix in their article to be fit for publication?

The current version of the manuscript needs a little more development before it is ready for publication. That authors make a few vague statements throughout the manuscript that, I feel, if they took some time to develop would be really influential to ecology.

Do you have any concerns about the ethics of this research?

No

Do you believe the article, in its current form, is fit for publication?

Revise and resubmit

Revised Submission

Do you have any conflicts of interest that could bias your ability to provide an independent review?

No

How well did the authors respond to your comments?

3/5

What - if any - feedback do you feel the authors did not adequately respond to?

There are still several places in the manuscript that need literature support through citations and the points they offer in the discussion to explain their results are still under developed.

Based on your review, what should happen next?

This paper needs major revisions and another round of peer review

Why is this article not ready to be published?

The authors added new concerns in their edits that need to be addressed

What do the authors need to change for you to accept this article for publication?

I am still skeptical of the overall takeaway and applicability of this study. Most of the discussion is focused on lipids, which was not a focus of this study. The difference between whole body and keratin structures is what was tested. And the ultimate conclusion of recommending a general -1.93+/- 0.37 is maybe irresponsible given that it could make a huge difference in diet estimates in other studies. For example, in my work we used published captive feeding studies that measured isotopic differences among tissues in bison, moose, caribou, and elk to estimate a TDF between moose hair and muscle. The difference between hair and muscle in these studies varied for δ13C by -0.08 to 1.14 ‰, and the ratios were (on average) 0.99, meaning that there was little difference in δ13C between hair and muscle in these species (Drucker et al., 2009; Milakovic & Parker, 2012). However, following the recommendation of this paper would shift my moose end-member by -1.93, which is half the total range of δ13C values for foods in our system, essentially increasing the width of the mixing space by 50%. That would drastically change my isospace and resulting diet estimates. I am not saying my study could not be improved by better incorporating the true isotope values of what consumers were eating but recommending that large of a shift would have huge effects on resulting diet estimates.

2nd Revised Submission

Do you have any conflicts of interest that could bias your ability to provide an independent review?

no

How well did the authors respond to your comments?
5/5
What - if any - feedback do you feel the authors did not adequately respond to?
there were a few odd comments that still need to be resolved
Based on your review, what should happen next?
This paper requires minor revisions but does not need further peer review
Would you like to be listed as a Collaborator on the final publication?
Yes, please list me as a Collaborator