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Interactive comment on "Sample preservation and pre-treatment in stable isotope analysis: Implications for the study of aquatic food webs" *by* Marc Jürgen Silberberger et al.

Anonymous Referee #1

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The ms by Silberberger et al. investigated the effect of different preservation methods (drying, freezing, formalin) on C and N stable isotope ratios in two marine invertebrate species, and the effect of acidification and lipid removal. The authors then apply Bayesian mixing models to determine the extent to which these sample prep methods affect the outcome of the mixing models.

While there are some valuable data in the manuscript, I fail to see the overall relevance of explicitly investigating how these sample prep differences translate into mixing model output. The point is that this will depend strongly on the 'absolute' values of the sources and consumers, and I don't see how general conclusions can be drawn. The

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authors mention that they also tested whether lipid and formalin corrections 'improved' the model results but there is no objective way to claim whether the output is better or worse then the original. "improved" implies closer to reality. Hence I do not see how we should interpret the authors' conclusions that (L408): 'the model outcomes are only rarely improved and equally often worsened' – the authors remain vague on how they interpret this.

If dried samples (and acidification for d13C if carbonates could be present) are the reference, then indeed all of the pre-treatment and preservation methods may or may not results in shifts in measured d13C or d15N values. A wealth of studies have assessed such changes, often with variable outcomes – but we have a good idea of the range of changes that can be expected. When subsequently applying a (Bayesian or not) mixing model, the key assumptions that need to be be made are regarding the trophic shifts for d13C and d15N. In my view, it would make sense to think about how these trophic shifts were determined – if those literature estimates were based on measurements with standard sample prep (no lipid extraction etc) then they need to be applied to similar data, if not then a correction to your sample data is needed in order to account for that.

If I consider the objectives of this manuscript: (i) quantify how sample preservation and pre-treatment affect carbon and nitrogen SI ratios (ii) identify potential interaction effects between preservation and pre-treatment methods (iii) study how preservation and pre-treatment affect the results of Bayesian mixing models (iv) assess whether lipid normalization and mathematical formalin correction should be used to adjust data for the use in such models.

then for me (i) and (ii) are fine, but (iii) and (iv) are not. I feel the ms should either forus on objectives (i) and (ii) but in that case it becomes a small dataset that is perhaps not sufficiently novel compared to the existing literature, or alternative think on expanding the scope – but I'm not sure that is feasible with the data at hand.

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Specific suggestions/comments:

The authors apply a ‘scaled trophic fractionation’ for d13C, whereby this is assumed to be large for the first trophic transfer (4 +/- 1.3 per mil) and smaller (0.4 +/- 1.3 per mil) for subsequent trophic transfers. Frankly, it is the first time I come across this, and if I follow the references this is based on, this all comes from a single paper (Hobson et al. 1995). I do not see much confirmation of the validity of this assumption in later reviews on trophic fractionation, e.g. Caut et al. (2009) Caut et al (2009) Variation in discrimination factors (Δ15N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology 46: 443-453. https://doi.org/10.1111/j.1365-2664.2009.01620.x

In particular for datasets such as the one the authors have, where C and N isotope ratios for most sources (pPOM, sSOM, ssSOM) are very similar and one is quite different (rPOM), the choice of the frationation factor has a very strong influence on the results, likely much more then the relatively small shifts induced by sample pre-treatment or storage.

There is also a large body of additional literature looking into the effects of lipid extraction and lipid corrections, e.g. the recent one by Cloyed et al. (2020) and references therein. Cloyed et al. (2020) The effects of lipid extraction on δ13C and δ15N values and use of lipidâĂŘcorrection models across tissues, taxa and trophic groups. Methods in Ecology & Evolution 11: 751-762. https://doi.org/10.1111/2041-210X.13386

Terminology:

-avoid the use of ‘stable isotope concentrations’ (L38), you’re looking at stable isotope ratios

-d13C values of different samples are higher or lower compared to each other, or they increase or decrease. Samples are enriched or depleted in 13C relative to each other. Avoid the use of mixed terminology such as ‘a depletion in d13C’ (L43 and throughout

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the ms), an ‘enrichment in d13C’ (L43 and throughout the ms).

-data corrections: in the methods (L131-133) the authors mention that data were corrected using pure reference gases calibrated against IAEA standards. This is not correct: the reference gases should not be used to calibrate the data, they merely serve as a monitoring gas, and data should be corrected using results of certified (or in-house calibrated) standards during the run. Perhaps this was done (I hope so) but it is merely not formulated properly.

-C/N ratios (L163 and further): to avoid confusion, please mention if these are weight/weight or molar ratios.

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