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 #2

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This study reports the effects of sample preservation and further treatment on stable isotope values in two marine species (Crustacea and Bivalva) and further consequences on the application of Bayesian mixing models to infer the relative contribution of several food sources. The text "Implications for the study of aquatic food webs" in the title seems to me too ambitious considering that the study only refers to two species. The first part of the study in well-presented and organized, providing interesting information on SI for the focussed species. The authors use different transformations and corrections on original Si values, depending on previous treatment of the samples. So many data manipulations seem rather confusing sometimes and will certainly affect the results achieved in the second part of the manuscript, focussed on mixing models. I

really do not understand the interest of comparing the author's data on SI values with those resulting from mathematical corrections according to Post et al. (2007) and McConnaughey & McRoy (1979). From my point of view and considering the huge work made on samples treatments, the original data provided in the study is of certain value in itself and will partially contribute to increase the knowledge on stable isotopes in marine organisms. The most interesting part of the study would have been that the authors provide mathematical corrections for the conversion between preservation methods for the objective species, which should be species-specific. About the second part of the study, there are several constrains. The first one refers to the values applied to trophic enrichment factors (TEF) for d13C and d15N. Generalist TEFs are useful when we do not know the actual TEF for a given species, which is the case of this study. However, in some cases those TEFs are unrealistic for a given species, e.g. Planas et al. (2020). (Effect of diet on breeders and inheritance in syngnathids: application of isotopic experimentally derived data to field studies. MEPS https://doi.org/10.3354/meps13315). Hence, the use of unspecific TEF values should not be used as reference for checking "improvement" of mixing model outputs. The use of the term "improvement" is another issue in this study. As the authors claim, an objective of the mixing models analysis performed was to assess whether normalization and mathematical corrections should be used to adjust data for the use in such models and to improve modelling results. Achieving an improved model imply a comparison with a control model, which is lacking in the study. The use of generalist TFEs do not ensure that model outputs are actual references for comparisons. Besides, mixing models provide relative contribution estimates of dietary sources. Ideally, overlapping significance of estimates should be analyzed (Bhattacharyya's coefficients) to assess the accuracy of source contribution estimates (Bhattacharyya, A., 1943. On a measure of divergence between two statistical populations defined by their probability distributions. Bull Calcutta Math Soc 35:99-109). Mixing models were carried out using a "long" run approach (which is not always sufficiently long as expected). Even though diagnostics (Gelman-Rubin and Geweke) might be fine with a long run, important differences in outputs might arise

sometimes when the model is submitted to an "extreme" run. Did the authors assayed any extreme run? If so, were there any differences compared to a long run? Finally, the use of isotopic "depletion" or "enrichment" is not correct when referring to values obtained from sample treatments. The terms "decrease" or "increase" must be used instead.

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