Dr Sebastian Naeher
Associate Editor, Biogeosciences
Biogeosciences

Dear Dr. Naeher,

Subject: Submission of revised manuscript bg-2022-170; "Endogenic methylmercury in a eutrophic lake during the formation and decay of seston" by Laura Balzer et al.

Thank you for handling the manuscript.
We have carefully revied the comments of the reviewers. Our responses are given in a point-by-point manner below. Changes in the manuscript are shown in a marked-up version of the manuscript.
The reviewers commented on our sampling technique and reviwer2 is concerned about the term “seston” and our sample content. According to him we have sampled only living plankton (with a 25µm plankton net) and he believes, that the term “seston” only comprises non-living particulate matter. We give a detailed point-by-point response below why the think that this is not correct and why we still want to use the term seston in our manuscript.
In short:
The use of plankton nets to sample seston is quite common in the literature (e.g. Yigiterhan et al., 2020, Biogeosciences).
In all references we found, including text books, seston is defined as all particles suspended in water regardless of their nature or origin including both, living plankton (phytoplankton, zooplankton, bacterioplankton, pseudoplankton, paraplankton) and detritus (dead biogenous, terrigenous, aerogenous and anthropogenous material).
Our seston samples contain a varying composition of plankton and detritus, with higher amounts of living plankton at the surface. Seston is therefore the best superordinate term to describe all our samples from the surface to the hypolimnion. The obvious change in colour of our samples from light green to light brown clearly indicates that the material does not only consist of living phyto- and zooplankton. Instead, the changing colour is mainly indicating decaying organic matter. If our samples would include only living biota all detritus must be < 25 µm which is unlikely. Moreover, if we do not see decay in our material, as suggested by reviewer2 we would not see oxygen depletion and formation of a RTZ.
The main idea of our study is to show how THg and MeHg concentrations and proportions change during organic matter decay in sinking seston in the water phase during algae blooms and this approach is new, to our knowledge. We suggested the formation of MeHg in seston micro niches as a possible process to explain the high methyl-Hg proportions in the samples of the upper water layers but we agree with the reviewer that we did not show this and it is not our point.

We have included a more detailed explanation why we have taken the samples in this way and a definition of our seston samples in our manuscript.
We have also replaced the term endogenic to “within the water column” or water column, respectively and modified the title of the manuscript which now reads “Role of formation and decay of seston organic matter for the fate of methylmercury within the water column of a eutrophic lake”.

We hope that the revised version is now suitable for publication and look forward to hearing from you in the near future

Sincerely,

Laura Balzer

Review 2

I understand that the aim of the paper is not to resolve the entire Hg cycle in the lake, but a focus on the role of the seston in Hg/MeHg. But then the sampling strategy to collect the seston with a 25 µm net excludes a portion of the seston. What proportion of the seston present in the water column is not sampled and analysed? An assessment of the implications of this partial(?) sampling would strengthen the interpretation of the results.

We agree with the reviewer that this sampling strategy of only sampling particles >25 µm is not so common in Hg analyses. All proportions of seston that are smaller 25 µm were not sampled (nano and pico plankton/particles).

Our goal was to study the temporal and spatial occurrences of lacustrine MeHg in settling particles at high resolution and how it changes during OM decomposition throughout the water column. Therefore, we needed sufficient material from each water layer to do the solid phase analyses to be able to show changes within 1 m of the water column. Sediment traps, for example, collect settling particles as a bulk sample integrating sedimentation over a specific time (days, weeks..) and the whole water column above. By sampling at one day in 1m interval directly from the water column we were able to cover daily lake fluctuations like stratifications that may change from hours to weeks. (Ortiz et al., 2015) showed that "methylation can occur as long as large particulates are present (>8 µm) [and that] it is unlikely that conditions conducive to methylation would occur in the smallest size fraction, which is likely composed of individual particles, small phytoplankton, and other microbes". Ortiz et al. 2015 concluded that methylation by anaerobes in oxic waters must be due to the formation of reduced oxygen microzones within the larger aggregations. As we said before, we tried a pump-and –sieve filter system before. However, this took too long to gain sufficient material from each water layer to do the solid phase analyses needed here at a resolution of 1 m within a single day (filter clogging etc., batteries etc.). Because of this, we decided to pump the water through a 25 µm net. In this way we obtained material that contains larger aggregates which could provide an ideal environment for mercury methylation because of the formation of anaerobic conditions. Although it would have been the best option to sample all phytoplankton fraction, we believe that the lack of the fraction < 25 µm has no significant influence of the overall results and conclusions of this study. Although we agree with the reviewer that nano and pico-plankton is involved in MeHg uptake and alteration (see Cossart et al., 2021)

We will add the following text for clarification (L104..)

"With the used method it was not possible to gain sufficient material from deeper layers in the water column, as the amount of suspended matter below 4-5 m was, in most cases, very low. [All seston samples were frozen immediately after sampling and subsequently freeze-dried and homogenized with a glass pestle for further analyses (THg, MeHg, CNS).]

Herein, we define seston as all particles suspended in the water column, including plankton (phytoplankton, zooplankton, bacterioplankton, pseudoplankton, paraplankton) and detritus (biogenous, terrigenous, aerogenous and anthropogenous detritus) (Lenz, 1977), larger in size than 25 µm. This method does not distinguish between the two types of seston nor

further between phyto- and zooplankton. Thus, our seston samples are a collection of varying compositions of plankton and detritus and their subgroups.

This approach excluded the pico- and nano-sized seston fraction (< 25 µm). We are aware that the smaller fraction is of importance within the microbial loop and would potentially extend our data. Pumping the water through the 25 µm net was the best method and within the range of our possibilities, which provided enough material for all solid analyses and allowed us a high sampling frequency (each water layer at a resolution of 1 m within a single day at several days a year) in the best of our abilities.Ortiz et al. (2015) showed that anoxic microinches can be formed within aggregations as long as the particles are larger than 8 µm. Smaller particles (composed of individual particles, small phytoplankton, and other microbes) do not provide ideal conditions for Hg methylation as no anoxic microniches can occur (Ortiz et al., 2015). Thus, we are confident, that seston >25µm size allow us to study the temporal and spatial occurrences of lacustrine MeHg in settling particles, how it changes during OM decomposition throughout the water column, to cover daily lake fluctuations like stratifications that may change from hours to weeks and to analyse if anoxic microniches may be formed also in shallow eutrophic lakes."

Review 1

Review of Balzer et al R1. See comments in red below. My recommendation for R1: Revisions inadequate. Reject Referee Review of Balzer et al. (2022): "Endogenic mercury…" General comments.

This paper builds on several prior studies that show that the water column of lakes and oceans can be an important site for MeHg formation. It differs from most water column studies by focusing on a eutrophic urban lake and by specifically targeting MeHg abundance in bulk seston at different depths and dates for clues about formation and decay mechanisms. Unfortunately, the sampling technique lumped zooplankton in with seston, potentially introducing bias due to biomagnification. And the sampling scheme was also spatially inconsistent, which makes the comparison of depth profiles on different dates difficult. The reason that the entire water column was sampled on one date and only the upper water column on most other dates is unexplained, and it compromises the authors' conclusions about what's going on as particles sink (especially in the hypolimnion since it was rarely sampled). Among other things (below), the authors need to justify their sampling methods and revisit the interpretation of changes in Hg speciation across depth and time. They also need to reconsider conclusions about links between climate change, productivity and bioaccumulation. This will require major revision.

Most of the issues raised above remain unresolved in the revised MS. The reason(s) that they sampled only the upper water column on most dates have not been given; and, in contradiction, they claim to have sampled the entire water column on 7 dates at 1m depth intervals. The interpretation of changes in Hg speciation across space and time continues to be largely speculative.

The reason why there is no data from below the RTZ in some of the profiles is that there was not enough suspended matter below the RTZ which could be sampled with our method (25 µm net several 2 hours pumping) (see L101-103).

We will add the following sentence: *"With the used method it was not possible to gain sufficient material from deeper layers in the water column, as the amount of suspended matter below 4-5 m was, in most cases, very low."*

In L 91-94 of the revised MS we wrote " Water and seston samples were taken on seven days between April and November 2019 using a clean stainless steel immersion pump (Comet Combi 12–4T). The water column was sampled over the deepest (~12 m) portion of the lake. Samples were collected from the surface down to the sediment water interface at 1 m intervals." We will change the last sentence to "*water* samples were collected from…. " to make clear that only the water samples has been collected from the surface down to the sediment.

Specific comments.

1. The term "endogenic" should be reconsidered. It means "within the system", which for lakes technically includes sediments. "Water column" would be better, unless they mean "within the seston" – in which case the title and text need to be re-worded. This term remains problematic. The authors refer to anoxic microniches in sinking particles as important sites for MeHg formation (e.g. "lake snow"), but the collection and analytical methods don't target these zones in dead suspended aggreagtes. Instead, they target live plankton that acquire MeHg by absorption or ingestion. That can't tell us anything about MeHg formation pathways in anoxic microniches or anywhere else in the lake .

We will change the term endogenic to "within the water column" or water column, respectively.
We will change the title „Endogenic methylmercury in a eutrophic lake during the formation and decay of seston" to:
"Role of formation and decay of seston organic matter for the fate of methylmercury within the water column of a eutrophic lake"

Regarding the term seston. See answer below.

2. Line 89 is an incomplete sentence

Has been corrected

3. Line 90: why a 25um net? It would allow many cyanophytes and chlorophytes to pass through, and bias collection toward zooplankton (which are not "seston"). Why not a clean pump-and-sieve/filter system instead? R1 L109: This question has not been resolved and it is a fatal flaw. "Seston" is the nonliving particulate matter in the water column, as opposed to "plankton" which is the live phytoplankton, nanoplankton and zooplankton. The sample collection method used in this paper would be strongly biased toward plankton. As living organisms, plankton do not have the anoxic microniches (generally attributed to "lake snow".) Instead, they often have defence mechanisms that prevent the accumulation of

microbes on their surface. In short, this paper does not directly address anything about anoxic microniches in lakes.

In all references we found, including text books, seston is defined as “all particles suspended in water regardless of their nature or origin. Depending on the aspect being dealt with, the particles can be classified under different headings, for instance according to particle size or chemical composition” (Lenz, 1977). Here is written that Seston can include both, plankton (including phytoplanklton, zooplankton, bacterioplankton, pseudoplankton, paraplankton) and detritus (including biogenous, terrigenous, aerogenous and anthropogenous detritus). We decided to use the term seston to include all the mentioned types of plankton and detritus, as our sampling method did not distinguish between the two types of seston nor further between phyto- and zooplankton. The relative contributions of plankton and detritus and their subgroups can differ significantly between samples and is hard to distinguish (Yigiterhan et al., 2020).(“There have been few studies that tried to distinguish the relative contributions of biotic and abiotic particles in marine particulate matter (Lam et al., 2015; Ohnemus and Lam, 2015; Ohnemus et al.,2017; Wen-Hsuan Liao et al., 2017)” from (Yigiterhan et al., 2020).) We assume that the samples from the surface contain higher amounts of living plankton than samples from the RTZ and the hypolimnion that contain higher amounts of dead detritus and abiotic particles. But we have not analysed the specific amounts of plankton or detritus in our samples. Seston is therefore the best superordinate term to describe all our samples from the surface to the hypolimnion. The obvious change in colour of our samples from light green to light brown clearly indicates that the material does not only consist of living phyto- and zooplankton. Instead, the changing colour is mainly indicating decaying organic matter. If our samples would include only living biota all detritus must be < 25 µm which is unlikely. The use of plankton nets to sample seston is quite common in the literature (e.g. Yigiterhan et al., 2020, Biogeosciences). Moreover, if we do not see decay (but only live plankton) in our samples, as suggested by the reviewer we would not see oxygen depletion and formation of a RTZ.

We will include the following sentences for clarification:

L 107-111“Herein, we define seston as all particles suspended in the water column, including plankton (phytoplankton, zooplankton, bacterioplankton, pseudoplankton, paraplankton) and detritus (biogenous, terrigenous, aerogenous and anthropogenous detritus) (Lenz, 1977), larger in size than 25 µm. This method does not distinguish between the two types of seston nor further between phyto- and zooplankton. Thus, our seston samples are a collection of varying compositions of plankton and detritus and their subgroups.”

Regarding the second point of the reviewer “anoxic microniches”:

It was shown in previous studies that anaerobic conditions can be formed in the centre of marine snow even if the aggregation contain photosynthetic active organisms (Alldredge and Cohen, 1987; Shanks and Reeder, 1993). Based on this, even if our samples contain living phytoplankton it is possible that they agglomerate with other particles to larger aggregates that provide an ideal environment for mercury methylation because of the formation of anaerobic conditions (Alldredge and Cohen, 1987). The model of anoxic microniches includes

the formation of MeHg by microbes within/ in the centre of the aggregated particles and not on their surface as commented by the reviewer (Alldredge and Cohen, 1987).

We will include the following paragraph for clarification:

"It was shown in previous studies that anaerobic conditions can be formed in the centre of marine snow even if the aggregation contain photosynthetic active organisms (Alldredge and Cohen, 1987; Shanks and Reeder, 1993). We did not distinguish the relative contributions of plankton and abiotic particles in our seston samples. We suggest that the seston samples from the surface contain higher amounts of living plankton than samples from the RTZ and the hypolimnion that contain higher amounts of dead detritus and abiotic particles as oxygen got depleted with depth. Anaerobic conditions are more likely to form the larger the particles and the less photosynthetically active the particles are (e.g. in the dark) (Alldredge and Cohen, 1987) "

4. L220-225. The seston samples collected on those dates are not really much closer to the sediment surface. There's just one hypo sample and it's directly beneath the RTZ. You'd need to sample more depths to justify. Revise. R1 L245-250. The small number of samples is still a serious limitation. Obviously, O2 depletion indicates that high rates of metabolically efficient decomposition have occurred. That's why there is an RTZ and anoxic hypo in the first place. There are no further insights into MeHg formation or demethylation in the data.

See comment above.

The main idea of our study is to show how THg and MeHg concentrations and proportions change during organic matter decay in sinking seston in the water phase during algae blooms and this approach is new, to our knowledge. We suggest the formation of MeHg in seston microniches as a possible process to explain the high methyl-Hg proportions in the samples of the upper water layers but we agree with the reviewer that we did not show this directly and it is not our point. We discussed also other possible explanations for the relatively high seston MeHg concentration in our manuscript.

5. L235. But peak concentrations of MeHg in seston occur in the suboxic RTZ on 4 of the 5 dates when the lake was strongly stratified. On the remaining date, seston MeHg concentrations are highest in the upper hypolimnion. During stratification, MeHg is never highest in the oxic epilimnion. If anything, these finding suggest that MeHg production is associated with microbial respiratory pathways that are less energy efficient than O2 reduction (e.g. sulfate reduction, Fe reduction). Revise. R1 L264-267. The revised text is better (more aligned with the data), but it now argues against their premise that methylation is occurring mainly in anoxic microniches within decaying seston as it settles. When the classic redox sequence has set up in the water column, the anaerobic microbes that possess the hgcAB genes can produce MeHg, but they don't have to reside within anoxic microniches in settling POM. Nothing in the data presented in this paper indicates or proves that they do. Instead, Mn, Fe and SO4 reducers may simply set up shop at the optimum depth and utilize the flow of nutrients and terminal electron acceptors from above (or below). This may occur in sediments or the water column. None of this is news, and none of it necessarily involves anoxic microniches in seston.

We cannot completely follow the reviewer here. The sentence in Line 235 of the original manuscript has been removed and the section has been clarified. It is not clear to which part the reviewer is referring his comment "..argues against their premise that methylation is occurring mainly in anoxic microniches within decaying seston as it settles". Our highest MeHg concentration in the seston is above the highest Mn concentration in the water column and far above the beginning of Fe reduction. Our data indicates that there is no sulfate reduction and thus no SO4 reducers within the water phase. Stratification omit mixing from the sediment and layers below the RTZ to layers above and into the RTZ. Thus, it is likely that there is another cause for the high MeHg concentration in the seston than Mn, Fe and SO4 reducers using terminal electron acceptors from the water phase.

Besides, to our knowledge there is only one paper that found the hgcA gene in manganese-reducing bacteria (Peterson et al., 2020), but their ability to actually methylate HgII still needs to be demonstrated.

6. L240-245. Alternatively, low MeHg during high productivity may reflect biodilution in the larger phytoplankton biomass (i.e. parental seston). Lacking sound data, one can't distinguish zooplankton bias from biodilution in microplankton, and neither necessarily point to sestonic microniches. Revise. I'm not convinced by the arguments in R1.

We don't know how to clarify this part based on this comment.

7. L255-263. They could also be explained by the presence of free-water microbes that possess the methylation gene pair hgcAB and occupy a region below the O/A boundary. DOM rather than POM could be their carbon source. Revise. R1 L308-324. This section remains highly speculative, and absent more rigorous investigation, alternative hypotheses can't be evaluated. The authors "assumption" that their explanation is the correct one isn't convincing

We agree with the reviewer that our hypotheses are speculative because we cannot completely prove them or exclude them with our data. But we discussed possible explanations aligned with our data. We conclude that the high MeHg concentrations at the RTZ could be explained by settling seston that aggregates within the RTZ. Settling particles such as this may form anoxic microniches, providing a thin vertical layer of high Hg methylation and biological activity, as suggested in other studies (Gascón Díez et al., 2016; Schartup et al., 2015; Ortiz et al., 2015; Gallorini and Loizeau, 2022).

8. L275-284. Sestonic MeHg in the 20% range is not atypical for unpolluted temperate lakes. What's unusual is the very low %MeHg in April. R1. L350-358. The mention of O2 fluxes into settling particles again assumes we are dealing with nonliving POM, but it's more likely that the "seston" collected in the plankton net comprises live organisms. The conflation of plankton with "lake snow" or dead POM aggregates is a conceptual problem throughout this paper

The reviewer is referring to L 315-320 in the revised manuscript. For the discussion of seston and living organisms in the particles see description above.

We propose, that the particles in the surface waters do not form aggregates (like in the RTZ) that are large enough to provide an ideal environment for mercury methylation in these high O_2 environment.

We will include the following paragraph in our manuscript:

"The conditions in the RTZ in Lake Ölper are favourable to form anoxic micro-niches, whereas at the surface layer, oxygen concentrations related to the photosynthetic activity of the plankton are assumingly too high and the size of the particles too small for the formation of anoxic micro-niches"

9. L346. Actually, this was first shown in Little Rock Lake, which is only 10m deep, and subsequently in many other lakes in this depth range. Not just deep oligotrophic lakes(but the eutrophic part may be right). R1. L346-347. This has not been addressed. The text remains unchanged and it seems like an attempt to oversell the novelty of this research.

We would like to include these references and lakes as they can improve our MS. Unfortunately, the reviewer missed to give us any reference. It is not clear for us to which paper the reviewer is referring to.

10. L346-end. Note that the range of Hg and MeHg in the seston of this eutrophic lake is on the low end of seston data reported for mesotrophic to oligotrophic North American lakes, both for MeHg concentration and %MeHg. High productivity is not necessarily conducive to abnormally high rates of MeHg accumulation in bioseston. In fact, most data suggest the opposite due to biodilution. It may be true that higher amounts of OM decomposition in eutrophic lakes does indeed exacerbate O2 depletion and enhance methylation in suboxic water, but that was not measured here. It seems that the most you can say with the data presented here is that the opposing forces of high biodilution and high decomposition need to be reconciled before addressing the impact of climate change. Revise R1 L349-357. This text also remains unchanged and it continues to promote the importance of anoxic microniches despite the fact that there is no direct evidence. The authors make claims about rates of methylation and demethylation without any rate determinations. Entirely unsupported speculations.

The main idea of our study is to show how THg and MeHg concentrations and proportions change during organic matter decay in sinking seston in the water phase during algae blooms and this approach is new, to our knowledge. We suggested the formation of MeHg in seston microniches as a possible process to explain the high methyl-Hg proportions in the samples of the upper water layers but we agree with the reviewer that we did not show this directly and it is not our point. We discussed other possible explanations for the seston MeHg concentration in our manuscript. However, our data suggest that free-water microbial Hg methylation is rather not the dominant process here as high MeHg concentration only occur during times of a pronounced RTZ (compare April when production is already high but MeHg is low because redox-zonation is not yet established).

We cannot follow the reviewer. We did not include any methylating and demethylating rate in our manuscript.

References

Alldredge, A. L. and Cohen, Y.: Can microscale chemical patches persist in the sea? Microelectrode study of marine snow, fecal pellets, Science (New York, N.Y.), 235, 689–691, doi:10.1126/science.235.4789.689, 1987.

Gallorini, A. and Loizeau, J.-L.: Lake snow as a mercury methylation micro-environment in the oxic water column of a deep peri-alpine lake, Chemosphere, 299, 134306, doi:10.1016/j.chemosphere.2022.134306, 2022.

Gascón Díez, E., Loizeau, J.-L., Cosio, C., Bouchet, S., Adatte, T., Amouroux, D., and Bravo, A. G.: Role of Settling Particles on Mercury Methylation in the Oxic Water Column of Freshwater Systems, Environ. Sci. Technol., 50, 11672–11679, doi:10.1021/acs.est.6b03260, 2016.

Lenz, J.: Seston and Its Main Components, in: Microbial Ecology of a Brackish Water Environment, Billings, W. D., Golley, F., Lange, O. L., Olson, J. S., Rheinheimer, G. (Eds.), Ecological Studies, Springer Berlin Heidelberg, Berlin, Heidelberg, 37–60, 1977.

Ortiz, V. L., Mason, R. P., and Ward, J. E.: An examination of the factors influencing mercury and methylmercury particulate distributions, methylation and demethylation rates in laboratory-generated marine snow, Mar. Chem., 177, 753–762, doi:10.1016/j.marchem.2015.07.006, 2015.

Peterson, B. D., McDaniel, E. A., Schmidt, A. G., Lepak, R. F., Janssen, S. E., Tran, P. Q., Marick, R. A., Ogorek, J. M., DeWild, J. F., Krabbenhoft, D. P., and McMahon, K. D.: Mercury Methylation Genes Identified across Diverse Anaerobic Microbial Guilds in a Eutrophic Sulfate-Enriched Lake, Environ. Sci. Technol., 54, 15840–15851, doi:10.1021/acs.est.0c05435, 2020.

Schartup, A. T., Balcom, P. H., Soerensen, A. L., Gosnell, K. J., Calder, R. S. D., Mason, R. P., and Sunderland, E. M.: Freshwater discharges drive high levels of methylmercury in Arctic marine biota, Proc. Natl. Acad. Sci. U.S.A, 112, 11789–11794, doi:10.1073/pnas.1505541112, 2015.

Shanks, A. L. and Reeder, M. L.: Reducing microzones and sulfide production in marine snow, Mar. Ecol. Prog. Ser., 96, 43–47, 1993.

Yigiterhan, O., Al-Ansari, E. M., Nelson, A., Abdel-Moati, M. A., Turner, J., Alsaadi, H. A., Paul, B., Al-Maslamani, I. A., Al-Ansi Al-Yafei, M. A., and Murray, J. W.: Trace element composition of size-fractionated suspended particulate matter samples from the Qatari Exclusive Economic Zone of the Arabian Gulf: the role of atmospheric dust, Biogeosciences, 17, 381–404, doi:10.5194/bg-17-381-2020, 2020.