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.

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 .
2. Line 89 is an incomplete sentence
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 defense mechanisms that prevent the accumulation of microbes on their surface. In short, this paper does not directly address anything about anoxic microniches in lakes.

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.
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.
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.
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
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

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.
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.