

## ***Interactive comment on “Dynamic mercury methylation and demethylation in oligotrophic marine water” by Kathleen M. Munson et al.***

**Kathleen M. Munson et al.**

kathleen.munson@umanitoba.ca

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### Author comment #2

Response to general comments: We authors agree with Referee #2's critique of our failure to properly emphasize the complexity of the changes in methylation and demethylation that occurred over the course of our incubations. Our desire, in fact, was to emphasize that 24 hour rate measurements fail to describe the complexity of methylation and demethylation processes that occurred during our incubations. We presented rate constants in Table 2 to show that despite the clear variability in net methylation observed (high initial methylation followed by rapid demethylation) in our incubations, calculated rate constants were not out of range of those presented previ-

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ously. Our focus during our revisions of the manuscript will be to recalculate methylation and demethylation from the time points measured and focus our discussion on those. We will include the rate constants in Table 2 to demonstrate that 24 hours is not an appropriate for quantifying accurate methylation and demethylation rates in all marine waters. It was certainly not for our stations.

Both referees recognized our failure to provide sufficient details on calculations of demethylation and the presentation of those data. This was a major deficiency of our initial manuscript and thus the major focus of our revisions.

Specific comments: Title: What is supposed to be conveyed by adding “Dynamic” to the title? Is there also a “lethargic” methylation? Or are you referring to the “Dynamics” of mercury methylation : : :? Given that both referees commented on our use of “dynamic” we will omit its use. “Dynamic” was used in contrast to “static” to indicate that 202Hg(II) can be methylated, demethylated, and methylated again (based on our time course at the 12 °S station).

L 76: terminology: rather than referring to “enriched isotope spikes” use “isotope enriched spikes. It is the spike that is enriched with isotopes not the other way around. Agreed.

L89: how exactly did you calculate ambient MMHg concentrations? What is meant by “correction for the added MM198Hg spike”? Changed to: “Estimates of MMHg at the 17 °N incubation depths were made from subtracting added MM198Hg spike contributions from the MeHg concentrations measured in initial timepoint incubations bottles (Table 1).” Because of the rapid demethylation observed in many bottles, this is only a very rough estimate of the concentrations and only provides an order of magnitude estimate at best.

L112: the concept of the “punches from McLane in situ pumps” requires more explanation. This appears to be lab lingo, which is incomprehensible to me at this point, though later on the authors shed a bit more light of what this likely means. Changed

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to include details that were originally provided in lines 259- : “Treatments of carbon (1 mM, as succinate), inorganic cobalt (500 pM), and filtered particulate matter collected from McLane in situ pumps (Munson et al, 2015) were added to triplicate bottles. Pump filters were subsampled using a 2 cm (ID) acid-cleaned polycarbonate tube with a beveled edge. The 2 cm subsamples were cut in half using ceramic scissors and one of these halves was added to each sample bottle for particulate amendments.”

L116: given that incubations were not performed at in-situ T, I am missing a discussion how this might have affected the outcome, since T changes alone could alter bacterial activity, leading to changes in steady state MMHg levels. The incubation temperatures were generally within a few degrees of the in situ temperature, except for the 12 °S station where we were unable to maintain the refrigerator “incubators” at sufficiently high temperatures. We will add discussion of this increase in temperature emphasizing that station and the deviations from in situ temperature as well as general temperature trends.

L145: it is an interesting concept to determine Hg(II) through direct ethylation. However, to be convinced that this is actually a viable method, I would require more QA/QC data, especially ethylation blanks. I would assume that reagents used in the methods carry some inorganic Hg background (buffers, acids, the ethylation reagent : : :) We have added the ethylation blank values in the revised manuscript. In general, the reagents carried a low but quantified THg blank that was primary due to the MQ water rather than the salts used. The ethylating reagent THg blank was below our detection limit.

L150: How are you determining a first order decay constant from at best two data points? I assume that the two points ( $t(0)$  and  $t(24)$ ) themselves carry considerable uncertainty. Given than the exponential relationship, this should translate in rather large uncertainty of the resulting linear relationship and rate constant. Even if this calculation was doable (which I somehow doubt), at the very least, you should provide an uncertainty estimate, which his suspiciously absent for  $K_d$  values of table 2, while

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on the other hand uncertainties for  $K_m$  are provided. Equally concerning, I can't find a single data point (in a table or on a graph) for measured MM198Hg levels before, during or after incubation. This needs to be provided in order to ascertain the conclusions drawn in this paper. The error estimates for  $K_d$  values are indeed large and were omitted as an oversight. As discussed in our response to general comments, we have provided the demethylation data and methods in support of our discussion.

L158: as mentioned earlier, I think it is misleading to claim that this experiment determined “rates”. Instead, it determined the net methylation that occurred over a 24 hour incubation period. In the absence of a time series showing a continuous change in concentration over time, I like challenge the idea that this dataset allows the calculation of rates, let alone rate constants. Here, the “rate” is obtained by drawing a straight line between two arbitrary points on the time axis. If the authors had chosen to incubate all samples for 6 hours, we would be facing very different “rates”. This is an important point. We believe this is one of the most important findings from our data set. However since Referee #2 felt it necessary to point this out, we acknowledge our need to emphasize this point more clearly. As mentioned in our response to the general comments above, we believe it is important to present the 24 hour “rate” measurements in Table 2 to show that “reasonable,” and misleading, values comparable to other published studies can be easily calculated from 24 hour data.

L210: are you sure that MMHg was indeed demethylated prior to  $t(0)$ ? Have you considered other loss mechanisms, e.g. adsorption to container walls? Did you try to determine the T198Hg concentration in these samples? If there was demethylation, leading to 198Hg(II), it should show up during a total Hg determination or in the diethylHg peak of the chromatogram. If absent, what does this say about the demethylation mechanism? Would that mean the product of the demethylation is 198Hg(0)? Is that possible? Where did the 198Hg isotopes go, if they are no longer detectable as MM198Hg? Analysis of refilled sample bottles suggested little loss of MM198Hg to the bottle walls (< 1 %). As the entire sample volume was used to perform the MeHg

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analysis, we could not analyze T198Hg from the same incubation bottles. Instead, we generally saw a strong 198Hg(0) peak. However, we did not attempt gaseous standard curves on the Tekran 2700 used for the analysis, so the 198Hg(0) peaks were not quantified.

L218: Be careful to not confuse “rates” with “rate constants”. Demethylation rates may be expressed as the % loss per day, but this is a rather unusual expression for a rate constant, which for first order processes, has the unit of d<sup>-1</sup> (per day). Why do you add “%” at this point? In this instance, the use of “constants” is a typo carried over from a previous version of the paragraph. Thus the units (% d<sup>-1</sup>) is correct and refers to rates and not rate constants.

L223: I agree with this concept, but I disagree in that the data obtained here are indeed “rate constants” instead, they are more net conversions over 24 h of incubation. Agreed. As discussed in our response to the general comments above, we do believe that Table 2 has its place in our manuscript since it shows that 24 h “rate constants” may mask the true rates of methylation and/or demethylation. However, our initial over interpretation of our 24 hour rate constants is unwarranted.

L243: given the absence of any actual data on MM198Hg concentrations, it is difficult to validate this conclusion. We understand. As noted, we have provided the missing MM198Hg methods and results to support our discussion of demethylation.

L259: this description of the “punch” should go to the methods section. We changed our description of the punches in the material and methods, as described in our response to L112 above.

L285; this is an intriguing observation. I'd be curious if this an experimental artifact and artificial or if this indeed points to environmental relevance for the methylation process. Certainly worth exploring in more detail. Agreed.

L294+296: “appears to be a dynamic process” what is “dynamic” in this process?

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Seems to be an unnecessary filler. Please, omit “dynamic”. We have omitted the use of “dynamic” as discussed in our response to the comments on the manuscript title.

L295: how did you calculate the rate of MM202Hg demethylation? Please, explain. Here the time course at 12 °S does in most cases warrant the use of ln(198MMHg) vs time and was used for the presented value. This will be clarified in the revision.

L297: I completely agree that the 24 hour incubations don't offer the resolution which would allow rate estimates with any certainty. This is not a critique of the experiment, but merely an observation. As mentioned earlier, these experiments require an educated guess about appropriate incubation periods and one only discovers after the fact, how good the initial guess really was. But rather than risking an overinterpretation of the data, the authors should rephrase their conclusions accordingly. Take into account the inherent limitations of this type of study. As noted in our response to general comments, we have restructured the manuscript to incorporate the use of methylation and demethylation (as % of spike) over incubation time periods rather than emphasizing a 24 hour rate.

L343: this observation is indeed puzzling. Can it have something to do with the acidification that is used to stop incubations? Acidification is a potential hypothesis for the rapid demethylation but is outside the scope of our study to determine. Acidification with H<sub>2</sub>SO<sub>4</sub> is stable for MMHg and MeHg determination from environmental matrices with varying DOM concentrations as well as standards, which suggests that the rapid loss of MeHg from our incubation samples (but not the pre-equilibrated spike) may warrant additional study.

L352: another unnecessary filler: “active” seems the wrong word here, unless there is also a “passive” methylation process. We have omitted this redundancy.

L369-366: where is this discussion coming from? I fail to connect the body of this research to hgc genes. As both referees suggested that this discussion was out of

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place, we will omit it from the revision. This observation was included because our measurements of enhanced methylation in filtered water are consistent with a lack of bacterial methylation as indicated from the *hgc* gene screening.

L367: how do you know that cellular methylation is not important, when you only determined net 24 hour methylation, rather than studying what is going on in the first 6 hours, were cellular processes may very well be important. But after 6 hours cells die (for whatever reason) and only appear to be unimportant (in the artificial setting of a closed 250 mL bottle). While this could be the case of unfiltered water incubations, in most cases we observed no enhanced methylation in unfiltered water relative to filtered water. The 250 mL is indeed an artificial environment, but the differences in methylation between filtered and unfiltered water, rather than the duration of methylation in unfiltered water, is the basis of our conclusion.

Figure 2: What is the difference between panel a+b and c+d? There is no legend for panels a+c. Was the concentration of the Hg(II) substrate determined (how?) or is this the nominal spike concentration? The figure captioned has been revised. The Hg(II) substrate was determined from the quantification of the diethylHg peak from the chromatogram as outlined in the methods. The identification of filtered (a+c) and unfiltered (b+d) results was not transferred when we adjusted the figure labels and is now included in the caption.

Figure 3: the chosen presentation makes it very difficult, if not impossible for most treatments to decide if concentrations after 24 hours are smaller or larger compared to the  $t(0)$  starting point. Agreed. As noted in our response to Referee #1, we have removed the lines and split the figure panels and resulting y axis scales to clarify.

Table 1: typo for THg of 17N CMX: 1:0. This is not a typo, but we will clarify. Because of water restrictions on the cast used to collect water at the 17 °N station, we had to mix water from 2 depths. Thus the presented concentrations “1:0.59” are the concentrations in the water from 120 m and 150 m. However, since this is confusing, we will

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change to “0.59-1.00” for clarification.

Table2: Why are there no uncertainty estimates for  $K_d$  values? The uncertainty estimates were not transferred into the submitted manuscript version. However, the more egregious error is likely the need for a better calculation of demethylation. Our revision focuses on the % of isotope tracer demethylated and the accompanying error in our calculations.

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