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

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Author comment #1

Response to general comments: We authors agree with the Referee #1's comments that the calculated potential methylation and demethylation rates over a 24-hour period fail to encompass the complexity of the changes occurring in the experiment. Our replicates and higher temporal resolution at the 12 °S station presented in the manuscript clearly revealed that rate measurements calculated from a single bottle and/or time point can mask variability that are important to recognize as isotopic tracers continue to be used to estimate Hg methylation and demethylation in further studies. We believe the variability seen in our experiments were important to recognize before comparisons

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Discussion paper

can be made between water bodies.

Both referees rightly pointed out that we provided too few details concerning our “rate” calculations, especially demethylation. This was a significant oversight in our initial submission and has been addressed in the subsequent revision in the following ways: -details of the calculations are included in the materials and methods sections, including differentiation between calculations used to distinguish between demethylation of 198MeHg and 202MeHg. -in response to comments from Referee #2, we have focused our data presentation and discussion on the % loss of MeHg rather than on calculated 24 hour demethylation rates since these often fail to describe our observations.

In addition, we have adjusted the introduction to clearly identify the following hypotheses that guided our experimental design: 1. If intracellular bacterial methylation were the primary source of MMHg to marine waters, we expected higher methylation rates in incubations of unfiltered water compared to filtered water from the equatorial Pacific. 2. If in situ methylation is the primary source of MMHg to the marine water column, we expect elevated rates of net methylation in oxygen minimum waters relative to chlorophyll maximum waters due to the relative concentrations between these depths. 3. If sinking particles provide Hg(II) substrate to oxygen minimum depths, we expect enhanced methylation in waters amended with additional particulate material.

Line 86 – should it be “Hg species” or “Hg form”? - consider throughout the manuscript. Total Hg, Hg(0), MMHg, and DMHg were analyzed as chemical species of mercury in seawater samples. Thus “species” is the correct terminology. I am not aware of the exact definition of a chemical “form.”

Line 112 –“ 1/2 ” would be nicer if spelled out, I think. (- > this is also in line 259) Changed.

Line 114 – Instead just “T0” perhaps it would read nicer if an additional description appear near e.g. T0 = time of the initial sampling or something like that. Changed to “timepoint of incubation onset (t0).”

Line 116 – How about “Equator” instead of “0_” ? Changed.

Line 117 – I am not sure why the incubation was set at the highest temperature setting? Please explain in text. Changed to: “Bottles from Stations 17° N, 8° N, and the Equator (0 °) were incubated in the dark for 24-hr in refrigerators maintained at temperatures closely matching the in situ temperatures from which the samples were taken (Table 1).”

Line 118 – Provide final concentration of sulfuric acid in seawater. Changed to “H2SO4 (0.5 % final volume).”

Line 123 – Apropos “generally low” – This give a feeling as if kinetics of DMHg degradation and even MMHg degradation in different seawaters were well established but the first one was measure in only one study thus far! Please revise this text. This is an important point. We have changed the text to reflect the fact that a single study has distinguished between Hg(II) to DMHg methylation, Hg(II) to MMHg methylation, and MMHg to DMHg methylation. We want to emphasize the point that our measured methylation or demethylation cannot be directly compared to the production or demethylation of MMHg and DMHg, if analyzed separately.

Lines 126-127 – Make it clear that this is an assumption – reword appropriately. With the changes to the paragraph in response to the previous line comments, we adjusted the paragraph to reflect that our measurements of Hg(II) and MeHg encompass any conversions between Hg(II), MMHg, and DMHg.

Line 131 - Provide more information about your Matlab script - is it freely available? Who has written the code etc. We wrote the MATLAB scripts and have included them in the supplemental section with an example calculation.

Line 136 – present your assumption about the amount of MM198Hg spike used as an internal standard. The internal standard used was MM199Hg, not MM198Hg. As a result, there is no assumption about the amount used for the internal standard. Instead,

the amount of MM199Hg added (25 fmol) was used to monitor the MeHg ethylation recovery.

Line 139 - You highlight the “potential rates” but you have also assumed the linear reaction, which is a far cry from what we see in your data. Again, clarity to how the rates were determined would be beneficial. Valid point. As pointed out by Referee #2 as well, we relied far too heavily on our 24 hour “rates” and, as discussed, provided too few details concerning our exact calculations. We have adjusted our “rates” to a more appropriate use of methylation and demethylation along the time course of our incubations (as % methylated and % demethylated) and our description of “rates” accordingly.

Line 146 – Do you mean that the recoveries were comparable? – This sentence is not clear to me. We used 202Hg(II) determined as diethylHg from the chromatograms of the Tekran 2700-ICPMS system to estimate the 202Hg(II) substrate in each incubation bottle. We observed quantitative recovery of Hg(II) from standard curves of THg analyzed using the Tekran 2700.

Line 148 – Do you decide to use the term “apparent” or “potential”? Decide to choose one. I actually think that “apparent” is a more appropriate. You would need to check throughout the manuscript. I think these rates are apparent based on specific experimental design and should be treated with a grain of salt. As mentioned in our response to general comments, we have adjusted our use of rates to clearly distinguish between our 24 hour rates and the much faster time scales of methylation and demethylaton observed or indicated in our experiments. In our initial submission, we generally used “potential” to refer to the fact that any isotope tracer studies assume identical availability of the added tracer Hg(II) or MMHg with that of in situ Hg(II) and MMHg. We used “apparent” to reference our measured rates are not “potential” since the 24 hour incubation time is inappropriate for our study. Our revisions now reduce our interpretation of the 24 hour rates.

Line 151-154 - This paragraph needs to be improved and more information is required as I already noted in my general comments. Agreed, as discussed in our response to your general comments.

Results and Discussion First paragraph reads like “material and methods” Agreed and have removed this section as it is repetitive.

Line 162 – Yay for triplicates! Agreed.

Line 165 - What exactly is the 1st order here? Fig. 1 doesn’t show rates. Again, maybe I am unsure because you have not really specified how the potential rates were calculated. If the publisher does not limit figures then why not include all the data – show results from all incubations? The first order approximation indicates the dependence on concentrations of MMHg and Hg(II) for demethylation and methylation. Studies to date have used the calculation of first order rate constants

Line 175 - You mean reactivation of dormant cells? Sessile doesn’t seem right in this context. Changed to “dormant.”

Line 177 - This sentence feel unfinished. Could you put a comma there and finish it off with something similar to this “therefore decreasing the likelihood of microbe-mediated Hg methylation”. We have added the recommended terminology.

Lines 182- 183 - You say: “: : :enhanced methylation in filtered water may dominate in Pacific water” but this is too general. Please be sure that this statement is only relevant to your study region. While our findings are not general to the Pacific, our stations encompassed regions that are more oligotrophic than other regions where rates have been measured. We have revised to be more specific to our oligotrophic stations.

Line 223 – Remove extra period. Removed.

Lines 237-244 - This paragraph is not coherent. The topic sentence is talking about the importance of demethylation but this is not developed any further. In the topic sentence specify differences in what. We adjusted the paragraph to reflect our evaluation

of how relative rates of methylation and demethylation contribute to measured MeHg concentrations in seawater. Our aim is to emphasize the fact that MeHg concentrations in seawater are currently attributed to differences in methylation. However, our findings suggest that demethylation is rapid and the specific controls on demethylation warrant further study.

Line 253 - If Fig. S3 is so important and it seems that authors use it to support their claims then why not include it into the main text? Also, this figure could use O2 information interpolated on top of the OCRR values. The OCRR data were calculated and presented in our previous manuscript (Munson et al, 2015) focused on the measured Hg species in this region, thus we feel that a reprint of those data are not needed for the main text.

Line 258 – in situ should be in italics Changed.

Line 266 - it should be "filtered particulate matter" as it was no longer in suspension. Changed throughout the manuscript.

Line 271 - This comment is for the caption in Fig. S2 – please change wording. For example: Concentration of Hg(II) as calculated by balancing measured dissolved Hg forms i.e. THg, MeHg, Hg(0) based on equation 1 in the main text. Changed to "Concentrations of Hg(II) as calculating from full dissolved Hg speciation measurements (THg, Hg(0), MMHg, and DMHg) throughout the cruise transect based on Equation 1 in the main text."

Equation 1 – where is the MeHg diss. From? Why isn't it presented here? Also, where are the other measured values from? Total Hg, Hg(0), MMHg, and DMHg were measured and presented in our 2015 manuscript as cited in the current manuscript. Those data are used here to calculate Hg(II), the assumed primary substrate for methylation, here using Equation 1 as presented. We adjusted the description to make that more clear.

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For the paragraph beginning at Line 270 - I think that the discussion here is poor and should be expanded. There have been studies discussing the issue of availability whether bioavailability or chemical availability/reactivity. As noted in our response to the general comments, we have adjusted our revisions to address our guiding hypotheses for this work As a result, we have adjusted our discussion of bioavailability, primarily citing the results of Schaefer and Morel, 2009, which informed the design of our amendments.

Line 280 – Wording here is awkward – please revise Changed to clarify: “Inorganic cobalt can limit the growth of sulfate-reducing bacteria, including known Hg methylators (Ekstrom and Morel, 2008). Although inorganic cobalt (Co) serves as the center of methylcobalamin, the co-factor implied in hgcAB-mediated Hg methylation (Parks et al., 2013), Co is not known to influence Hg methylation. The significant increases in methylation from the additions of succinate and Co to oxygen minimum waters at 12 °S (Fig. 2) may indicate either a direct role of Co or a role of methylcobalamin in methylation. However, since the enhancement occurred in filtered waters rather than unfiltered water the enhanced methylation in the presence of succinate and Co in the presence of minimal cellular material warrants further study as a potential abiotic mechanism of Hg methylation.”

Line 282 – Again, please revise wording “additive additions” ??? That doesn’t sound right. Changed to “multiple amendments.”

Line 292 - Isn’t C succinate? If so I would just provide that name and remind the reader that it was a generic source of carbon. Changed.

Line 296 - What exactly do you mean by “dynamic methylation” – it sounds scientific but it delivers no meaning. Please consider changing it. You can simply describe the pattern of how and when things changed, just the way you did it in the second sentence. The use of dynamic was critiqued by both referees. As a result we have omitted its use in our revised manuscript. However, since we observed rapid methylation, rapid

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demethylation, and rapid shifts between methylation and demethylation, we continue to focus our discussion on the rapid turnover between Hg species observed in our incubations.

Line 317 – But the release of Hg(II) during the two processes i.e. sinking and remineralization are connected because enhanced microbial processes are associated with sinking particles that when nearer to surface are more organic matter rich. Indeed, sinking and remineralization of particulate matter are related. However, the distinction we aimed to make here is between the importance of "residence time" of Hg(II) in the water column that appeared to promote methylation in the Heimbürger et al, 2011 study of oligotrophic waters and the delivery of particulate matter to low oxygen depths that has been observed by Sunderland et al, 2009 and Cossa et al, 2009 hypotheses. The assumption that remineralization in low oxygen water

Lines 330 - till conclusions – great discussion on the shortcomings of spiking. Ligands need to be addressed in future research. Kept in revised manuscript.

Line – 360 – this is the first I read about any effort to identify genes from Metzyme- this comes out of nowhere. The whole issue of genes here is completely unexpected – I don't see how this fits as a conclusion to this particular paper. I recommend rethinking the conclusion. We were not involved with the gene identification. These "hgcA-like" genes were observed by Podar et al, 2015 from sequences of samples collected at our incubation stations. However, the identification of these genes similar to those that encode methylation, but not containing regions that contribute to methylation, is interesting in light of the fact that bacterial methylation is commonly cited as the primary mechanism of water column methylation. The enhanced methylation we observed in filtered compared to unfiltered water incubations is consistent with

Fig. 3 – I would get rid of all the lines – they blur the figure, which already contains a lot of symbols. Perhaps you can consider splitting these two panels into more small panels? - it would show patterns more easily and then you can keep lines connecting data

for specific time points. Scale on y-axis in a) is too large. I would increase resolution of the x-axis. We have adjusted the figure to more easily distinguish the methylmercury at each time point, especially in the chlorophyll maximum.

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