Dynamic mercury methylation and demethylation in oligotrophic marine water

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Abstract

Mercury bioaccumulation in open ocean food webs depends on the net rate of inorganic mercury conversion to monomethylmercury in the water column. We measured significant methylation rates across large gradients in oxygen utilization in the oligotrophic central Pacific Ocean. Overall, methylation rates over 24 hour incubation periods were comparable to those previously published from Arctic and Mediterranean waters despite differences in productivity between these marine environments. In contrast to previous studies that have attributed Hg methylation to heterotrophic bacteria, we measured higher methylation rates in filtered water compared to unfiltered water. Furthermore, we observed enhanced demethylation of newly produced methylated mercury in incubations of unfiltered water relative to filtered water. The addition of station-specific bulk filtered particulate matter, a source of inorganic mercury substrate and other possibly influential compounds, did not stimulate sustained methylation, although transient enhancement of methylation occurred within 8 hours of addition. The addition of dissolved inorganic cobalt also produced dramatic, if transient increases in mercury methylation. Our results suggest important roles for non-cellular or extracellular methylation mechanisms and demethylation in determining methylated mercury concentrations in marine oligotrophic waters. Methylation and demethylation occur dynamically in the open ocean water column, even in regions with low accumulation of methylated mercury.

1 Introduction

Mercury (Hg) is a toxic metal present in small amounts in the ocean that accumulates in fish to reach levels that pose human and environmental health risks. Because methylated Hg (collectively abbreviated as MeHg) accumulates more efficiently than inorganic Hg, (Hg(II)), transformations into MeHg ultimately control burdens in upper trophic level biota. These methylated species, monomethylmercury (MMHg) and dimethylmercury (DMHg), can be found throughout the water column, with concentration maxima often found at depths of rapid oxygen consumption (Mason and Fitzgerald, 1993; Cossa et al., 2009; Sunderland et al; 2009; Cossa et al., 2011; Bowman et al., 2015; Bowman et al., 2016). At these depths, production of methylated Hg has been linked to active organic matter remineralization (Cossa et al., 2009; Sunderland et al., 2009). Water column measurements of apparent Hg methylation rates in the Canadian Arctic Archipelago and the Mediterranean have attributed water column methylation to microbial processes (Lehnherr et al., 2011; Monperrus et al., 2007). However, the mechanism of methylation in these marine systems has not been determined.

Studies of obligate anaerobes from anoxic sediments have shown that Hg methylation is encoded by a pair of prokaryotic genes, *hgcA* and *hgcB* (Parks et al., 2013), the identification of which has revealed novel potential environments of methylation, such as sea-ice (Gionfriddo et al., 2016), and methylation capacity across a greater diversity of microbial species (Gilmour et al., 2013; Gilmour et al., 2018). Analysis of microbial metagenomes from our 8 °N (150 – 800 m) and the Equatorial (50 m) stations identified sequences similar to microbial *hgcA* sequences encoding methylation but containing base pair substitutions, the effects of which on methylation potential are unknown (Podar et al., 2015). These findings, combined with a lack determination of *hgcAB* distributions in marine waters to date, demonstrate that the contribution of microbially mediated methylation pathways, such as *hgcAB*, to inventories of MeHg in oxic marine waters is still unknown.

We measured Pacific water column Hg methylation and MeHg demethylation rates using tracer level stable isotope additions to examine potential sources of the MeHg maxima in low oxygen waters. We anticipated that both methylation rates and demethylation rates would differ between waters with generally low MeHg concentrations found at the chlorophyll a maximum depths and the generally elevated MeHg concentrations found at the oxygen minimum depths. We also hypothesized that MeHg concentrations in

low oxygen waters could be limited by organic matter supply. Thus, we expected that the addition of organic matter would promote net methylation. Alternatively, if methylation is limited by microbial activity, we expected that the stimulation of remineralization by carbon addition could enhance net methylation.

In order to gain insight into the mechanism of Hg methylation in the marine water column, we tested these hypotheses across gradients in concentrations of MeHg, total dissolved Hg (THg), dissolved oxygen, and apparent oxygen utilization (AOU) (Munson et al., 2015) in waters from the central Pacific Ocean collected between Hawai'i (17 °N) and American Samoa (12 °S) during the "Metzyme" cruise. Across this region, low oxygen intermediate waters centered at 12 °N set apart regions of higher MeHg concentrations (~260 fM) in intermediate waters (Sunderland et al., 2009) from regions of low (<100 fM) concentrations in the central and south Pacific, with the exception of relatively elevated concentrations centered around the Equator (Munson et al., 2015). The cruise track included regions of the North Pacific where intermediate waters incorporate anthropogenic Hg emissions (Sunderland et al., 2009; Lamborg et al., 2014) and marine fish Hg content has increased in recent decades (Drevnick et al., 2015).

2 Materials and Methods

Stock solutions of isotope enriched spikes, (MMHg: ~225 µM; Hg: ~500 uM), were prepared from isotopically enriched HgO (Oak Ridge National Laboratory) and stored at 4°C until use. Isotopically enriched $MM^{198}Hg$, for methylation rate measurements, and $MM^{199}Hg$, used as an internal standard during analysis, were prepared from ^{198}HgO and ^{199}HgO, respectively, by methylation with methylcobalamin (Hintelmann and Orinc, 2003). A mass of 100 µg of enriched HgO was dissolved in 10 µL HCl (conc, J. T. Baker) and diluted in 500 µL sodium acetate buffer (0.1 M, pH 5). A mass of 500 µg of methylcobalamin (Sigma) was dissolved in 500 µL sodium acetate buffer and added to the Hg solution. The reaction proceeded for 3 hours at room temperature before being stopped with 200 µL KBr (0.3 M KBr in 2 M H_2SO_4). The enriched MMHg was extracted with toluene (400 µL, 3x). Extracts were combined and dried over sodium sulfate. A 100 µL aliquot of this primary stock was dissolved in 10 mL isopropanol to produce a secondary stock solution. Carryover of methylcobalamin as Co in the stock solutions was found to be minimal (<1 pM) from ICPMS analysis (Thermo Element 2).

Water for incubation experiments was collected on board the *R/V Kilo Moana* between 1–24 October 2011 (Table 1). Water was collected from two depths at each station: 1) the chlorophyll a maximum and, 2) the oxygen minimum identified from the SeaBird CTD package data on prior water sampling casts at each station (Table 1). Water was collected in acid-washed X-Niskin bottles deployed on a dedicated trace metal sampling rosette using Amsteel metal-free line. Water filtration, decanting, and treatment additions were performed under positive pressure from HEPA filter laminar flow hoods within clean bubbles constructed from plastic sheeting. Concentrations of dissolved Hg species (Table 1) were measured throughout the cruise transect (Munson et al., 2015), with the exception of 17 °N, where no MMHg samples were preserved. Estimates of MMHg at the 17 °N station incubation depths were made by subtracting $MM^{200}Hg$ contributions in isotope spike additions from the MeHg concentrations measured in initial timepoint incubation bottles.

Incubation water was decanted under N_2 pressure either unfiltered or filtered through a 0.2 μm capsule filter (47 mm, Supor polyethersulfone membrane, Pall Corporation) into 20 L acid-washed polycarbonate mixing carboys (Nalgene). The carboys were covered in dark plastic bags during incubation set-up to minimize exposure to light. Although hard to quantify, cells have been found in marine waters after filtration through 0.2 μm filters in the Mediterranean and Sargasso Seas (Li and Dickie, 1985; Haller et al., 1999) under vacuum filtration. As a result, we cannot exclude the possibility that small cells, such as the SAR11 clade (Rappé et al., 2002), viruses, and cell material passed through filters and therefore was present in our filtered incubations. However, 0.2 μm filtration would potentially collect a subset of intact cells such as SAR11 due to clogging of filters and decreases in actual filter pore size. In addition, if the cells that pass through filters are dormant or, like SAR11, have slow growth rates (0.40—0.58 d^{-1}) (Rappé et al., 2002), filtered waters would likely have lower cell density and less metabolic activity compared to unfiltered waters.

Pre-equilibrated spikes of isotopically enriched $MM^{198}Hg$ and $^{202}Hg(II)$ were prepared by adding concentrated $^{202}Hg(II)$ and $MM^{198}Hg$ to 0.2 μm filtered seawater and equilibrating at 16°C in the dark for 24 hours prior to use. Spikes were added using gas-tight syringes dedicated for use in isotope enrichment experiments (Hamilton).

Incubations were performed in triplicate in 250 mL amber borosilicate glass bottles (I-Chem) filled to the shoulder from mixing carboys leaving approximately 25 mL of headspace. As a result, in situ redox conditions were not maintained during incubation. After water addition, pre-equilibrated spikes of ^{202}Hg(II) (396 fmoles) and MM^{198}Hg (126 fmoles) were added. Thus, spike concentrations were added at approximately 1–14x ambient Hg(II) and 1–34x ambient MMHg concentrations. 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 (punches) were cut in half using ceramic scissors. One half of a subsample was added to each sample bottle for particulate amendments.

Bottles for the initial timepoint of incubation (t0) were preserved after isotope spike and treatment addition with 1 mL (0.5%, final) H_2SO_4 (conc., Fisher TM grade) and were stored at -4 °C until analysis. Bottles from Stations 17 °N, 8 °N, and the Equator (0 °) were incubated in the dark for 24 hr at temperatures maintained in refrigerators adjusted up to their highest temperature settings to most closely match in situ temperatures (Table 1). Station 12 °S bottles were incubated in the dark up to 36 hr in a time course study. After incubation, bottles for all time points were preserved with H_2SO_4 (0.5 %, final) and stored at -4 °C. Because DMHg decomposes to MMHg in acidic conditions (Black et al., 2009), our analysis measured the sum of DMHg and MMHg present at in each bottle at the time of acidification. A previous study of methylation rate measurements in waters from Arctic Canada have distinguished between production of MMHg and DMHg from Hg(II) as well as the conversion of MMHg to DMHg (Lehnherr et al., 2011). Rate constants of methylation to DMHg from Hg(II) were measured between 0.03–1.22 % those of MMHg production from Hg(II) (Lehnherr et al., 2011). However, in the same study, conversion of MMHg to DMHg proceeded more rapidly and rate constants ranged between 3.17–43.3 % of those measured for MMHg production from Hg(II) (Lehnherr et al., 2011). In contrast, the rates presented here represent MMHg production from Hg(II) substrate and include any MMHg subsequently converted to DMHg.

Samples were analyzed as for MMHg using ascorbic-assisted direct ethylation (Munson et al., 2014), which generates volatile methylethylmercury from the sample analyte. Analysis of MMHg and

Hg(II) concentrations and isotopic composition was performed on a ThermoFinnagan Element 2 ICPMS in the Plasma Facility at the Woods Hole Oceanographic Institution linked to a Tekran 2700 Automated Methyl Mercury Detector (Tekran, Ontario, Canada). An internal $MM^{199}Hg$ standard (25 fmol) was added to samples and equilibrated for 24 hours prior to MMHg determination to account for differences in ethylation efficiency between samples.

Hg isotopes were measured individually and relative counts were integrated using MATLAB scripts (provided in Supplement) to quantify isotopic signals in the MMHg (as methylethylHg) and Hg(II) (as diethylHg) peaks. Resulting methylation of $^{202}Hg(II)$ and demethylation of $MM^{198}Hg$ were quantified using a matrix inversion approach to solve the system of equations (Hintelmann and Ogrinc, 2003). Methylation rates were calculated from the observed ingrowth of $Me^{202}Hg$ divided by $^{202}Hg(II)$ substrate supply and incubation time. Significant $Me^{202}Hg$ production was observed in t0 incubation bottles. As a result, the reported methylation rates were not calculated from the difference between final and initial time points (e.g. t24 hr minus t0) as this often yielded a negative value indicating demethylation of $Me^{202}Hg$ between time points. Previous reports of methylation rates have differed in their considerations of available Hg(II) substrate, including assuming a constant supply of Hg(II) substrate (Monperrus et al, 2007) and an exponential model of decreasing Hg(II) substrate availability throughout the course of an incubation (Lehnherr et al, 2011). Generalized loss of Hg(II) was not an accurate representation of Hg(II) availability in our experiments. Although not analytically identical to total Hg measured by tin chloride reduction followed by CVAFS analysis (Lamborg et al, 2012), we observed quantitative recovery of Hg(II) from the diethylHg peak of the Tekran 2700 instrument used for CVAFS analysis (p values <0.05 from daily standard curves). Due to the presence of Hg(II) in the laboratory water used for reagent preparation, averages of Hg(II) peak integrations from reagent blanks (5–7 daily) were subtracted from those used to calculate daily standard curves. Sequential additions of reagents to filtered seawater (1x, 2x, and 3x reagent volume) did not change Hg(II) peak integrations after baseline correction. Results are presented as percentages of $^{202}Hg(II)$ substrate that was methylated in each incubation bottle. Apparent methylation rate constants, k_m, were calculated as the percentage of $^{202}Hg(II)$ substrate methylated over the incubation time.

For stations 17 °N, 8 °N, and 0 °, demethylation rates were determined from differences between measured $MM^{198}Hg$ at incubation time points (t24 minus t0) divided by the initial $MM^{198}Hg$ addition (126

fmole) and incubation time. At station 12 °S, demethylation rates of both added $MM^{198}Hg$ spike and newly produced $Me^{202}Hg$ were measured from the time course experiments. These rates were calculated assuming first order kinetics from the fit of exponential decay of the methylated Hg form versus incubation time (Prism 7, GraphPad).

Consistent with previous reports of methylation and demethylation rates (Monperrus et al, 2007, Lehnherr et al, 2011), our reported rates are potential rates and assume identical chemical behavior of added Hg(II) and MMHg isotopes to those species naturally present in the samples.

3 Results and Discussion

3.1 Unamended Samples

Following 24 hour incubation periods at the 17 °N, 8 °N and Equatorial stations, we calculated first-order methylation and demethylation rates. Methylation rates were enhanced in 0.2 μm filtered water relative to unfiltered water (Fig. 1) with the exception of low oxygen waters at the Equatorial station. The observation of methylation in filtered seawater was unexpected given recent focus on the role of bacterial pathways analogous to those that occur in anoxic sediments (Monperrus et al., 2007; Lehnherr et al., 2011). However, abiotic mechanisms of methylation have been identified from experiments conducted in distilled water and non-marine natural waters (Yin et al., 2014; Celo et al., 2006). Because the incubation waters we collected were influenced by the biota present prior to filtration, we do not distinguish between truly abiotic and biologically influenced mechanisms that occurred within these waters. Furthermore, although filtration may not remove all cells (Li and Dickie, 1985; Haller et al., 1999) we do not attribute the enhanced methylation observed in filtered waters to cellular mechanisms. Such an assumption would require a number of processes, such as reactivation of dormant cells, rapid rates of cell growth, and/or preferential selection of Hg(II) methylating cells during filtration. Instead, we assume that filtration removed a majority of heterotrophic bacteria relative to unfiltered waters therefore decreasing the likelihood of microbially mediated methylation in filtered samples.

Similar experiments in Mediterranean waters reported significant methylation in waters filtered through 0.45 μm filters, which allows the passage of some plankton and many microbes relative to unfiltered waters (Monperrus et al., 2007). Our methylation rates measured in filtered waters averaged 1.4x

those of unfiltered waters (range 0.58-2.1x). This is opposite to the methylation rates measured in filtered Mediterranean waters (0.8 % d^{-1}), which were half those in unfiltered waters (1.7 % d^{-1}; Monperrus et al., 2007). Thus, enhanced methylation in filtered water may dominate our central Pacific station depths due to its nutrient regime while larger bacteria and perhaps eukaryotic algae may contribute in more nutrient-rich environments such as those studied by Monperrus et al. Furthermore, the relative role of non-cellular versus microbial methylation may change in contrasting marine regions or due to seasonal variability. Direct comparisons cannot be made until additional rates are determined in filtered water.

In addition to enhanced methylation in filtered waters, non-cellular methylation is indicated by rapid initial methylation of added ^{202}Hg(II) spike within the elapsed time, 30–120 minutes, between ^{202}Hg(II) spike addition and preservation of the t0 samples with acid (Supplement Fig. S1). This initial methylation, like that of methylation rates over 24 hour incubations, was highest at 8 °N (subsurface chlorophyll maximum (CMX) filtered water: 1.98 % d-1), the Equator (CMX filtered water: 2.50 % d^{-1}; oxygen minimum (OMZ) filtered water: 1.17 % d^{-1}) and 12 °S (CMX unfiltered water: 1.14 % d^{-1}), where it accounted for all methylation observed during the 24 hour incubations (Supplement Fig. S1). Rapid methylation has been observed in measurements of labeled Hg(II) additions to lake water (Eckley and Hintelmann, 2006), and in previous incubations of unfiltered seawater (Lehnherr et al., 2011). Previous marine methylation rate measurements with isotopes have exclusively used unfiltered waters or filters that allow microbes to pass through (Monperrus et al., 2007; Lehnherr et al., 2011), which prevented distinction between microbial and non-microbial methylation in those experiments.

Methylation rates were decoupled from ambient MeHg concentrations. At chlorophyll maximum depths, methylation rates were highest at the Equator (CMX: 2.03–2.56 % d^{-1}) and lowest at 17 °N in the North Pacific (CMX: 0.69 % d^{-1}; Fig. 1) despite nearly identical ambient MeHg concentrations (Table 1). At oxygen minimum depths, methylation rates also exhibit now relationship with measured MeHg concentrations, including 17 °N in the North Pacific (OMZ: 0.74–1.10 % d^{-1}), where the methylation rate was lowest despite the highest MeHg concentrations (Table 1). As a result, methylation rates alone do not account for observed MeHg concentrations across gradients of productivity.

Previous dual tracer measurements have quantified simultaneous methylation and demethylation within marine waters (Monperrus et al., 2007; Lehnherr et al., 2011), which yield the persistence of MeHg

species. Net methylation is indicated when MeHg concentrations predicted from steady-state methylation and demethylation rate measurements account for more than 100 % of ambient MeHg. Conversely, net demethylation is indicated when predicted MeHg concentrations account for less than 100 % of ambient MeHg. However, rapid demethylation of the added $MM^{198}Hg$ spike prior to t0 fixation was observed in the majority of our samples, which yielded non-quantitative demethylation rates in many cases (Table 2) due low excess MMHg concentrations (median: 10–47 fM from all stations) remaining at measured timepoints despite $MM^{198}Hg$ spike additions in excess of 500 fM (full data in Supplement). At the 12 °S station, higher resolution timepoint measurements yielded (Table 2) quantifiable measurements of $MM^{198}Hg$ resulting in demethylation rate constants that were an order of magnitude higher (5.9 and 7.8 d^{-1}) than those measured in Arctic waters (0.23–0.59 d^{-1}; Lehnherr et al., 2011). These limited measurements and the demethylation that is indicated by the loss of $MM^{198}Hg$ spike prior to t0 fixation suggest the importance of demethylation despite our inability to quantify demethylation rates.

3.2 Amended samples

The relative importance of methylation and demethylation on steady-state MeHg concentrations were further explored by testing whether methylation rates were limited by Hg(II) substrate supply or co-factor supply in central Pacific waters. The correlation between MeHg concentrations and rates of organic carbon remineralization in North Pacific Intermediate Water (Sunderland et al., 2009) indicates that these low oxygen waters are poised for MeHg production from Hg(II) substrate released during remineralization. The oxygen minimum depths targeted at each station corresponded to depths of highest rates of organic matter remineralization (Supplement Fig. S3). However, unamended methylation rates were lowest at 17 °N along the cruise transect despite water from this station displaying the greatest drawdown of dissolved oxygen (AOU: 283 μmol kg^{-1}; Table 1).

We amended incubation water with filtered particulate matter, carbon (as succinate), and cobalt additions. The filtered particulate matter punches were collected from oxygen minimum depths at each station using in situ pumps (Munson et al., 2015) to test its ability to increase methylation through the supply of Hg(II) substrate, thus testing whether methylation in the water column is limited by Hg(II) substrate availability. Each punch represented the particulate matter in approximately 70 L of seawater, a

~310 fold enrichment of particles to the incubation and 0.35 to 1.05 pmol of added total Hg (Table S2). Succinate was added to distinguish between general stimulation of microbial activity and the site-specific particulate matter contained in the filter punch additions.

Rather than relieving widespread Hg(II) limitation in low oxygen waters, the addition of the punch material only stimulated methylation in filtered waters from the oxygen minimum waters at the Equator over a 24 hour incubation period (Fig. 2C). The addition of filtered particulate matter to unfiltered waters from other oxygen minimum depths did not produce clear responses and high variability was seen between replicate samples (Fig. 2). Furthermore, the addition of filtered particulate matter lowered methylation rates relative to unamended samples over 24 hour incubation periods. The failure to stimulate methylation with site-specific organic matter suggests that methylation at these Pacific station depths is not limited by Hg(II) substrate availability. Full Hg speciation measurements at incubation stations (Table 1; Munson et al., 2015) can be used to calculate the maximum concentration of the dissolved Hg(II) species available as methylation substrate according to Eq. (1):

$$[Hg(II)]_{dissolved} = [THg]_{dissolved} - [Hg(0)]_{dissolved} - [MeHg]_{dissolved} \quad (1)$$

Concentrations of Hg(II) calculated from Eq. (1) are relatively high (~1 pM, Supplement Fig S2) in low oxygen waters at all incubations stations and comprise 41-90 % of the total Hg measured. These Hg(II) concentrations provide further support that methylation is not limited by Hg(II) substrate supply. Thus, neither bulk ambient Hg(II) concentrations nor increased local particulate matter supply appear sufficient as sources of Hg(II) substrate to the mechanisms involved in methylation in these waters.

Although the addition of filtered organic matter did not enhance methylation, amendments with other potential co-factors were more robust. Inorganic cobalt can limit the growth of, and MeHg production by, sulfate-reducing bacteria, including known Hg methylators (Ekstrom and Morel, 2008). Although cobalt serves as the center of methylcobalamin, the co-factor implied in *hgcAB*-mediated Hg methylation (Parks et al., 2013), inorganic cobalt (Co) by itself is not known to influence Hg methylation. As a result the significant increases in methylation from the combined addition of succinate and Co to oxygen minimum waters at 12 °S (Fig. 2) were surprising given that the greatest increase in methylation (2x that of

unamended water) occurred in filtered, rather than unfiltered water (Fig. 2C) where we hypothesized their addition would stimulate *hgcAB*-mediated methylation. 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, such as potential competition between Co(II) and Hg(II) for organic ligand binding that could increase Hg(II) substrate availability for methylation.

3.3 Time course

Although methylation rates calculated from 24 hour incubation timepoints (Table 2) were comparable to those measured in other marine regimes, higher temporal resolution of sampling at the South Pacific station, 12 °S, reveals that methylation of added ^{202}Hg(II) is rapid, with maximum methylation reached within 6 hours in unfiltered waters (Fig. 3). Likewise, increased methylation in amendments with C, Co, and organic matter was rapid (Supplement Table S2). Subsequent demethylation of newly produced Me^{202}Hg occurs within 12 hours of total incubation time in incubations of unfiltered water (Fig. 3). Demethylation appeared to be a dynamic process, with the demethylation rate constant of newly produced Me^{202}Hg (from added ^{202}Hg(II)) in unfiltered waters calculated at 1.8 d^{-1} in unfiltered water from the oxygen minimum depth (Fig. 3).

A transition between net methylation and net demethylation were observed within the first 12 hours of incubation in both chlorophyll maximum and oxygen minimum waters (Fig. 3). This rapid transition observed at 12 °S suggests that 24 hour incubation time points are not sufficiently resolved to document these reactions in detail at our level of spike addition in central Pacific oligotrophic waters. This implies that our calculated rates (Table 2) underestimate maximum rates of methylation and demethylation that occur in situ.

Demethylation in oxygen minimum waters from 12 °S over the entire 36 hour incubation period was influenced by the presence of particles, as shown by the demethylation of with newly produced Me^{202}Hg (Fig. 3). In contrast, methylation in filtered waters is similar in magnitude to that in unfiltered waters but persists over the course of the incubation (Fig. 3). The use of dual tracers in this time course experiment reveals that demethylation controls MeHg concentrations as the incubation approaches steady-

state, indicating an important role of demethylation in determining MeHg concentrations in the marine water column and its availability for bioaccumulation.

The rapid transition between MeHg production and demethylation that was observed in water from 12 °S obscures the conclusions that can be made from 24 hour incubation results (Fig. 1). Although amendment of incubated waters appeared to decrease methylation in comparison to unamended waters (Fig. 2), rapid demethylation of newly produced $Me^{202}Hg$ within the 24 hour incubation period could produce similar results. Notably, the low MeHg production that was indicated after 24 hours in unfiltered water (Fig. 2B, 2D) and filtered water (Fig. 2A, 2C) amended with filtered particulate matter could represent rapid methylation followed by demethylation kinetics similar to those observed at 12 °S.

3.4 Mercury Methylation and Organic Matter Remineralization

Although elevated ambient MeHg concentrations have long been recognized as occurring at depths of high oxygen utilization (Mason and Fitzgerald, 1993; Cossa et al., 2009; Sunderland et al; 2009; Cossa et al., 2011; Bowman et al., 2015; Bowman et al., 2016), gaps persist in our understanding of how organic matter remineralization influences methylation. While low MeHg concentrations in surface and deep waters are maintained due to demethylation by both photodemethylation and biological demethylation (Cossa et al., 2009; Sunderland et al., 2009; Monperrus et al., 2007; Lehnherr et al., 2011) subsurface peaks in MeHg concentrations are thought to be controlled by two factors: 1) the release of Hg(II) substrate from sinking organic matter remineralization (Cossa et al., 2009; Sunderland et al., 2009), and 2) enhancement of microbial loop activity (Heimbürger et al., 2010). Microbial loop activity has been suggested to dominate subsurface MeHg dynamics especially in oligotrophic waters (Heimbürger et al., 2010).

The subtropical gyre waters at 17 °N and 8 °N were oligotrophic regions exhibiting nitrogen stress while 12 °S was within a region exhibiting both low nitrate concentrations and iron stress (Saito et al., 2014). Concentrations of THg in the North Pacific reveal a sharp transition between North Pacific Intermediate Water and intermediate waters south of 17 °N (Sunderland et al., 2009; Munson et al., 2015). Within the Equatorial Pacific we observed an area of low Hg species concentrations. We also observed significant reduction of Hg(II) centered at 12 °N and extending toward the 8 °N incubation station that corresponds to regions of denitrification between 200–400 m, as indicated by calculated N* values

(Munson et al, 2015). The reduction of Hg(II) appears to limit the supply of Hg(II) to depths below 400 m, consistent with conceptual models of methylation due to substrate supply (Sunderland et al., 2009; Lehnherr et al., 2011).

However, the addition of filtered particulate matter from in situ pump filters to bottle incubation did not enhance potential methylation in waters collected from this site. These observations suggest that delivery of Hg(II) by bulk organic matter does not limit MeHg production in North Pacific waters. This observation is consistent with some field data, where ratios of MeHg to Hg below the photic zone is not elevated compared to other regions of the water column (Sunderland et al., 2009; Hammerschmidt and Bowman, 2012), including regions adjacent to our incubation stations (Munson et al., 2015). In contrast, the addition of Co and additive additions of Co and succinate were able to promote methylation, most notably in South Pacific waters, which suggests a more specific control, such Hg ligand binding or co-factor availability, on methylation rather than bulk Hg(II) supply. Therefore, the dominance and rapidity of initial methylation in our incubations may indicate the importance of ligand exchange with Hg(II) substrate in oligotrophic waters.

In addition to the potential role of organic matter in controlling Hg methylation, our results indicate an important role of particulate matter in demethylation and thus water column MeHg concentrations. The enriched Hg species spikes used as tracers were pre-equilibrated in filtered seawater for 24 hours prior to addition to bottle incubations in order to allow Hg to bind with DOM complexes in an attempt to more closely mimic seawater speciation of Hg (Lamborg et al., 2004). Given that the $MM^{198}Hg$ enriched stock solution was stable over the 24 hour pre-equilibration period in filtered seawater, the rapid demethylation observed upon addition of the spike to seawater at tracer levels in incubations was surprising. As the filtered seawater used for pre-equilibration differed from incubation water only in its ratio of enriched Hg to ligands, the rapid demethylation may further indicate the role of ligands in controlling Hg species dynamics.

4 Conclusions

We measured methylation of added $^{202}Hg(II)$ substrate in filtered waters, which suggests a potential role for non-cellular methylation in oligotrophic marine systems. Enhanced Hg methylation in the

central Pacific Ocean filtered water, implicates non-cellular or extracellular processes as the primary mechanisms of mercury transformation in the marine water column. Determining the extent of this non-cellular methylation relative to microbial methylation is important for understanding the potential for MMHg entry into the marine food web.

The potential for a non-cellular pathway of Hg methylation dominating at these Pacific water depths is consistent with current genomic studies. Although functional *hgcA* and *hgcB* genes have been identified in marine regions that are influenced by continental shelf sediments, permafrost, and estuarine waters, functional *hgcAB* sequences have not isolated from our incubation stations (Podar et al., 2015). The failure to identify functional *hgcAB* sequences from our incubation stations does not exclude the possibility that these genes are present in such low abundance as to escape detection or the ability of the uncharacterized sequences to encode methylation. However, our results suggest that regional differences in methylating co-factors, perhaps ligands and competing metals, and efficiency of particle driven demethylation may be more important than *hgcAB*-mediated methylation in controlling MeHg concentrations in the oligotrophic Pacific.

Although we expected increased methylation following stimulation of heterotrophic bacteria following succinate addition, cellular methylation did not appear to dominate oligotrophic waters along our Pacific transect. Instead, the high methylation rates in our incubations of filtered water suggest a need to investigate the role of Hg-ligand binding in controlling steady-state MeHg concentrations. Methylation over 24 hour incubations was enhanced by the addition of Co, potentially due to the activity of ligands those that can interact with both Hg(II) and Co. Ligands may also influence demethylation, which appeared to control MeHg concentrations across wide gradients in productivity across this section of the Pacific. Rapid demethylation was observed in filtered water, despite pre-equilibration, and following particulate matter addition. Demethylation, and thus the persistence of MeHg in the water column, may be influenced by the relative concentration of MeHg to organic matter, both dissolved and particulate.

Author contributions: KMM, CHL, and MAS designed research; KMM, CHL, and RMB performed research; KMM and CHL analyzed data; KMM and CHL prepared the manuscript with contributions from RMB and MAS.

The authors declare that they have no conflict of interest.

Acknowledgements

This work was funded by the National Science Foundation in a Chemical Oceanography Program Grant (OCE-1031271) awarded to CH Lamborg and MA Saito and a Graduate Student Fellowship to KM Munson. We thank Vic Polidoro, Trevor Young, Captain Drewry, the scientific and ship's crew of the *R/V Kilo Moana*.

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Station	Depth (m)		Temp (°C)	$Temp_{inc}$ (°C)	O_{2diss} (µmol/kg)	AOU[a] (µmol/kg)	THg (pM)	MeHg (fM)
17 °N	CMX	120 :150[b]	22	19-26	214:205	-	1:0.59	41
	OMZ	500	7	13	20	282.9	1.66	349
8 °N	CMX	75-80	21	23	218	-	0.32	31
	OMZ	200	11	13	15	264.1	1.4	152
0 °	CMX	50	26	23	202	-	0.18	36
	OMZ	500	8	14	37	252	1.1	255
12 °S	CMX	60	28	13-16	206	-	0.22	15
	OMZ	175	24	13-16	162	51.7	0.35	23
	OMZ	400	11	13-16	116	163.1	0.67	81

Table 1: Water column characteristics for Pacific Ocean waters from which potential mercury methylation rates were measured.

[a]AOU is a poor proxy for oxygen utilization in waters above 100 m due to gas exchange and photosynthesis. Values are therefore omitted for these depths.

[b]Water was combined due to water budget limitations and water was a 1:3 mixture from 120 m and 150 m depths.

[c]MMHg concentrations were not measured directly and were calculated from ambient $Me^{200}Hg$ in t0 incubation bottles by subtracting MMHg spike contributions.

Station	Depth (m)	k_m-0.2 µm filt ($x10^{-2}$ d^{-1}) ± 1 SD	k_m-unfilt ($x10^{-2}$ d^{-1}) ± 1 SD	k_d-0.2 µm filt ($x10^{-2}$ d^{-1}) ± 1 SD	k_d-unfilt ($x10^{-2}$ d^{-1}) ± 1 SD
				Linear calculations	
17 °N	CMX	0.43 ± 0.11	0.43 ± 0.02	0.02 ± 0.09	nd
	OMZ	0.95 ± 0.08	0.64 ± 0.05	0.06 ± 0.40	nd
8 °N	CMX	1.58 ± 0.27	0.91 ± 0.11	0.02 ± 0.53	0.12 ± 0.29
	OMZ	4.21 ± 2.31	0.63 ± 0.71	nd	0.06 ± 0.10
0 °	CMX	2.19 ± 0.20	1.74 ± 0.37	nd	0.03 ± 0.71
	OMZ	1.00 ± 0.19	1.74 ± 0.47	0.06 ± 0.18	nd
12 °S	CMX	0.69 ± 0.28	0.72 ± 0.02	-	-
	OMZ	0.20 ± 0.04	0.49 ± 0.08	0.34 ± 0.17	nd
	OMZ	2.28 ± 0.70	1.12 ± 0.21	-	-
				Exponential decay ($MM^{198}Hg$) (d^{-1}) ± SE	
12 °S	CMX			7.8 ± 6.7	nd
	OMZ			nd	5.9 ± 7.2
				Exponential decay ($Me^{202}Hg$) (d^{-1}) ± SE	
12 °S	OMZ				1.8 ± 0.99

Table 2: Potential methylation (k_m) and demethylation (k_d) rate constants from triplicate bottle incubations of Tropical Pacific waters in filtered and unfiltered water. Linear calculations were made from differences between t0 and t24 hour timepoints of added MM198Hg. Exponential decay was calculated from 36 hour time course incubations.

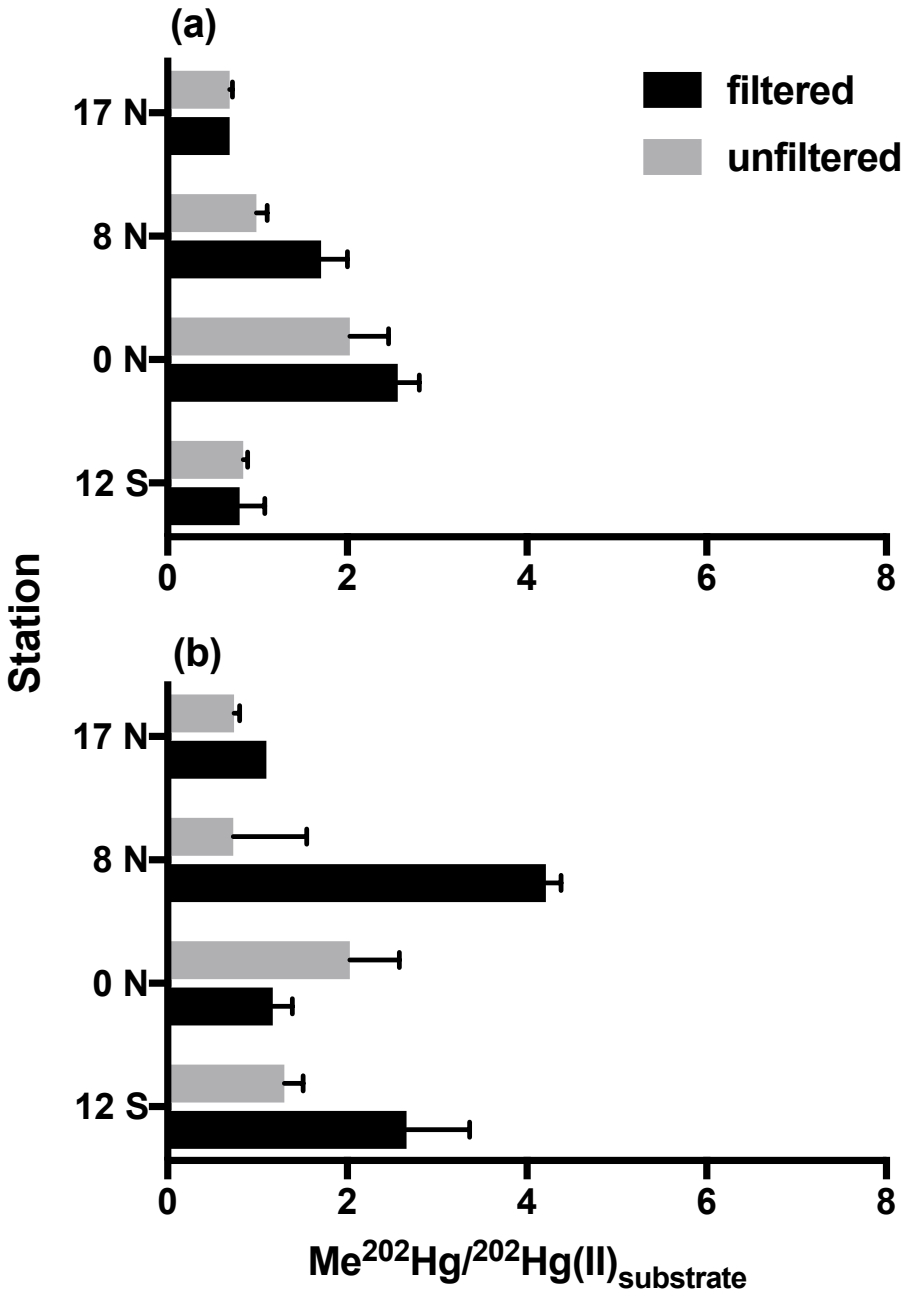
Figures Captions:

Figure 1: Methylation of Hg(II) in Tropical Pacific waters from triplicate bottle incubation (± 1 SD), as the percentage of $Me^{202}Hg$ produced from excess $^{202}Hg(II)$ substrate from isotope spike additions, for cruise stations from the North to South Pacific. Methylation was measured in 0.2 μm filtered and unfiltered waters from the chlorophyll maximum (a) and oxygen minimum (b) depths at each station.

Figure 2: Normalized methylation rates from 24 hour incubations. Methylation rates from triplicate incubation bottles (±1 SD) normalized to unamended (un) 0.2 μm filtered (a, c) and unfiltered (b, d) water from the chlorophyll maxima (a, b) and oxygen minima (c, d). Treatments are abbreviated as follows: cobalt (Co), succinate (C), in situ pump subsamples (punch).

Figure 3: Pacific water column methylated mercury (MeHg) production over 36 hours at 12 °S. Methylation rates from triplicate incubation bottles (±1 SD) in 0.2 μm filtered and unfiltered water from the chlorophyll maximum and oxygen minimum depths in the South Pacific. Rapid demethylation of MeHg occurred in the presence of particles in unfiltered low-oxygen water. Newly produced MeHg only persisted beyond 12 hours in 0.2 μm filtered water.

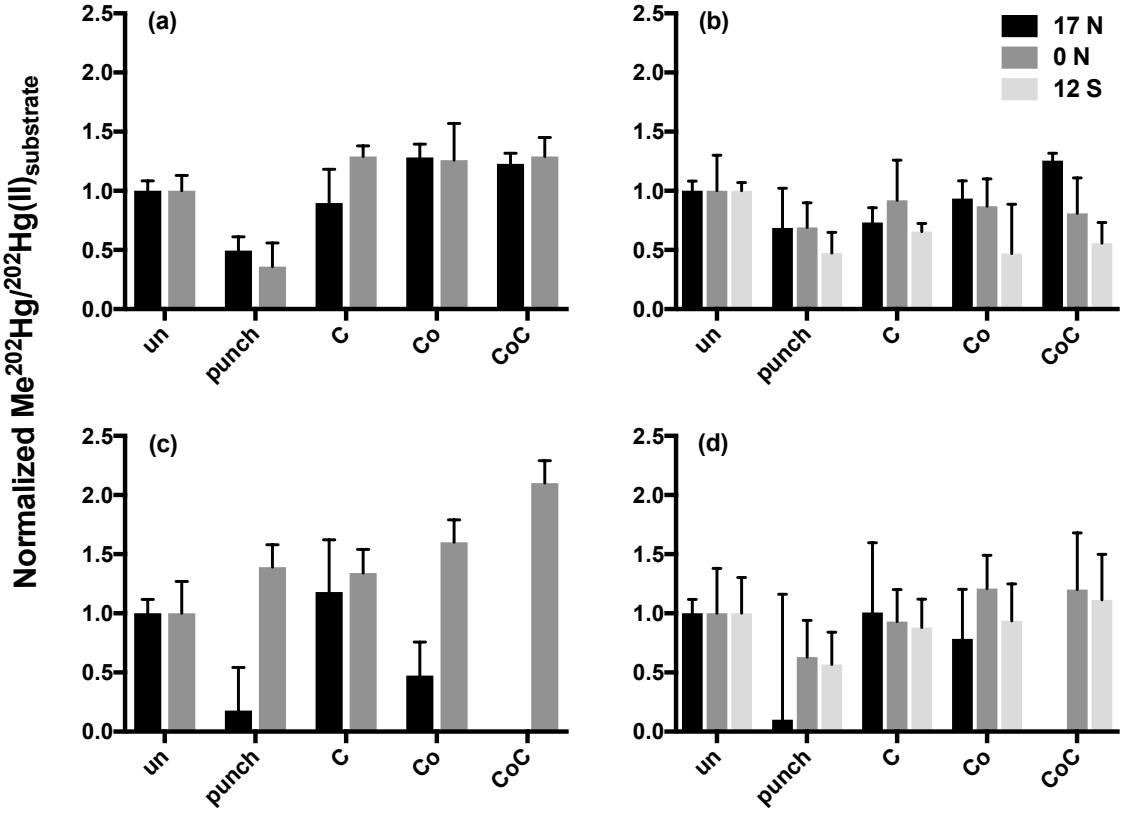
Figure 1:



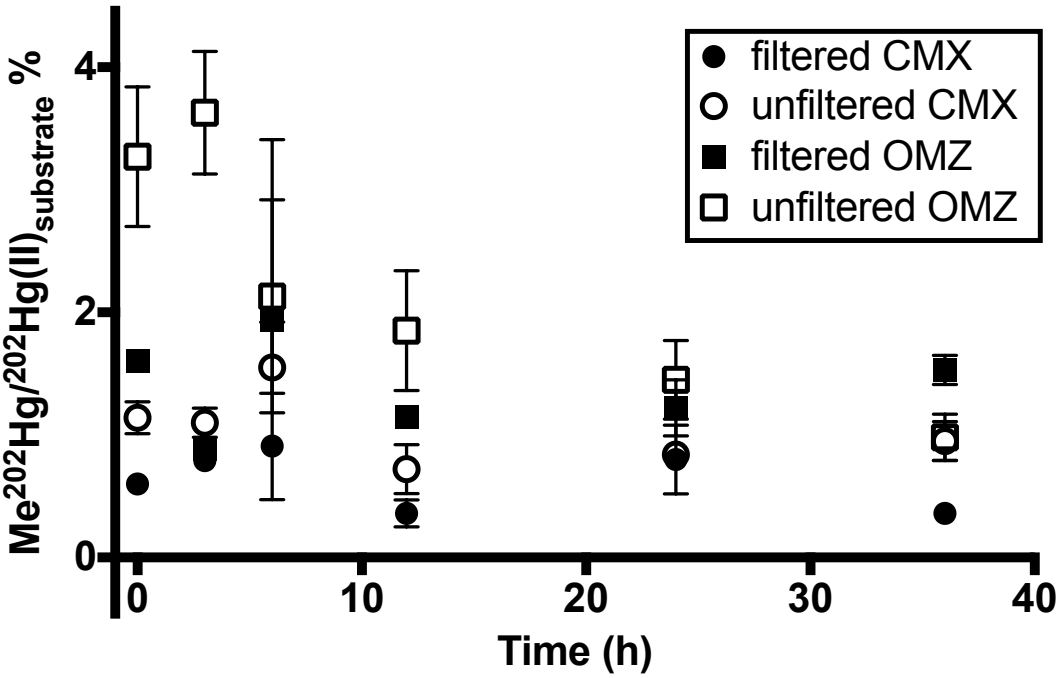
565

570

Figure 2:


Normalized Me202Hg/202Hg(II)substrate
(a)
(b)
(c)
(d)
17 N
0 N
12 S
2.5
2.0
1.5
1.0
0.5
0.0
un
punch
C
Co
CoC

575 Figure 3:


filtered CMX
unfiltered CMX
filtered OMZ
unfiltered OMZ
$Me^{202}Hg/^{202}Hg(II)_{substrate}$ %
0
2
4
0
10
20
30
40
Time (h)