Microbial cycling of mercury in contaminated pelagic and wetland sediments of San Pablo Bay, California

M.C. Marvin-DiPasquale · J.L. Agee · R.M. Bouse · B.E. Jaffe

Abstract San Pablo Bay is an estuary, within northern San Francisco Bay, containing elevated sediment mercury (Hg) levels because of historic loading of hydraulic mining debris during the California gold-rush of the late 1800s. A preliminary investigation of benthic microbial Hg cycling was conducted in surface sediment (0–4 cm) collected from one salt-marsh and three open-water sites. A deeper profile (0–26 cm) was evaluated at one of the open-water locations. Radiolabeled model Hg-compounds were used to measure rates of both methylmercury (MeHg) production and degradation by bacteria. While all sites and depths had similar total-Hg concentrations (0.3–0.6 ppm), and geochemical signatures of mining debris (as ϵNd, range: –3.08 to –4.37), in-situ MeHg was highest in the marsh (5.4±3.5 ppb) and ≤ 0.7 ppb in all open-water sites. Microbial MeHg production (potential rate) in 0–4 surface sediments was also highest in the marsh (3.1 ng g^{-1} wet sediment day^{-1}) and below detection (<0.06 ng g^{-1} wet sediment day^{-1}) in open-water locations. The marsh exhibited a methylation/demethylation (M/D) ratio more than 25× that of all open-water locations. Only below the surface 0–4-cm horizon was significant MeHg production potential evident in the open-water sediment profile (0.2–1.1 ng g^{-1} wet sediment day^{-1}). In-situ Hg methylation rates, calculated from radiotracer rate constants, and in-situ inorganic Hg(II) concentrations compared well with potential rates. However, similarly calculated in-situ rates of MeHg degradation were much lower than potential rates. These preliminary data indicate that wetlands surrounding San Pablo Bay represent important zones of MeHg production, more so than similarly Hg-contaminated adjacent open-water areas. This has significant implications for this and other Hg-impacted systems, where wetland expansion is currently planned.

Keywords Demethylation · Estuary · Mercury · Methylation · Wetland

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Introduction

From 1852 until 1884 the practice of hydraulic mining of soft ancestral river deposits was widespread in the Sierra-Nevada mountain region of the western US (Alpers and Hunerlach 2000). Elemental mercury (Hg^0) was used in recovering gold and silver from the resulting ore-slurry. Over 2.0 billion m^3 of placer gravel was processed in this manner with much of the mercury-contaminated debris subsequently transported downstream towards the San Francisco Bay (SFB). Because the highest deposition rates occurred over a fairly narrow and discrete time frame, this historic mining debris is often evident as a distinct Hg enriched horizon in SFB sediment cores (Bouse and others 1996). In some Bay areas, this layer is being buried, while in others it is being re-exposed because of net sediment loss or dredging. A recently developed model, which predicts the occurrence and depth of this debris layer, was used to guide sampling of debris and non-debris areas in San Pablo Bay, a 282-km^2 estuary within northern SFB. Methylmercury (MeHg) is a biological neurotoxin, which is produced from inorganic Hg(II) primarily in anoxic sediments, and is readily accumulated in aquatic food webs. Interest in the mechanisms that mediate MeHg production in the environment has increased significantly in recent years because of human-health implications associated with the consumption of Hg-contaminated fish. It is unknown to what extent the Hg-enriched hydraulic mining layer in SFB may influence MeHg production rates. Do higher Hg levels, associated with this layer, necessarily result in higher rates of MeHg production? Alternatively, does the depth of the debris layer, relative to the zone of maximal microbial activity ultimately mediate the degree

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of MeHg production? To explore these questions, total-Hg (Hg_t) and MeHg content, hydraulic mining debris geochemical signature (as ϵNd) and microbial potentials for both MeHg production and degradation were examined in surface sediment of San Pablo Bay. The study's two primary objectives were to (1) examine how the presence or absence of the hydraulic mining material impacts microbial Hg-transformations, and (2) determine the relative importance of open-water versus salt-marsh sediments as zones of MeHg production. This latter objective is particularly important in light of the current efforts to reclaim wetland areas in the San Francisco Bay-Delta region, in an attempt to improve overall ecosystem health (CALFED 1999). A mounting number of ecosystem studies indicate that wetlands are important zones of MeHg production (Olson and Cooper 1976; Branfireun and others 1996; Driscoll and others 1998; Gilmour and others 1998; Naimo and others 2000). Subsequently, while wetland reclamation may bring many positive benefits (e.g., increased wildlife habitat), increasing the spatial extent of these regions in San Pablo Bay, and similar systems, may also increase net MeHg production.

Materials and methods

Sediment sampling

Sediments from three open water (2.4–3.3 m depth) locations (sites 1–3) in San Pablo Bay (Fig. 1) were collected by box cores (20×30×50 cm) during March 2000. Subsamples were taken using polycarbonate core rings (4 cm height × 8 cm i. d.) and stainless steel spatulas. Additional sediment was collected in the intertidal pickleweed (*Salicornia virginica*) zone (no overlying water) of a salt-marsh on the southwest edge of the bay (site 4). This marsh was formed between 1856 and 1887, when massive quantities of hydraulic mining debris were deposited in San Pablo Bay (Gilbert 1917; Jaffe and others 1998). Surface sediment (0–4 cm) was collected at each location, and a deeper profile (0–4, 8–12, 16–20, 22–26 cm) was collected at site 3. Two 0.5-l glass mason jars were filled to capacity for each site/depth for microbial assay experiments. Samples were stored in double zip-lock bags in coolers containing in-situ temperature bottom water, and subsequently refrigerated at 5 °C until further use. A separate 5–10-cm^3 subsample was transferred to a fluoropolymer container, which was immediately stored on dry ice and remained frozen until further Hg-speciation analysis. All containers and sampling equipment was acid cleaned in 3 N HCl and rinsed with ultra-clean distilled deionized water prior to use. Fresh sampling gear was used at each site. Fluoropolymer containers used for Hg-speciation samples were further pre-cleaned by hot acid reflux (USEPA 1996). Sites were selected based on a reconstructed bathymetry model (Jaffe and others, in preparation), which predicted the following conditions with respect to the location of historic hydraulic mining debris deposits: Site 1 – control site, no debris; site 2 – mining debris in surface sediment; site 3 – mining debris layer approximately 10 cm below sediment surface; and site 4 – marsh site, mining debris present. Sediment redox (E_h), pH, and temperature measurements were taken at the time of sample collection using a Cole–Parmer pH/mV/Temperature meter (model #: pH 20) adapted with a platinum redox electrode (model #: 5990-55) or a temperature compensating pH electrode (model #:

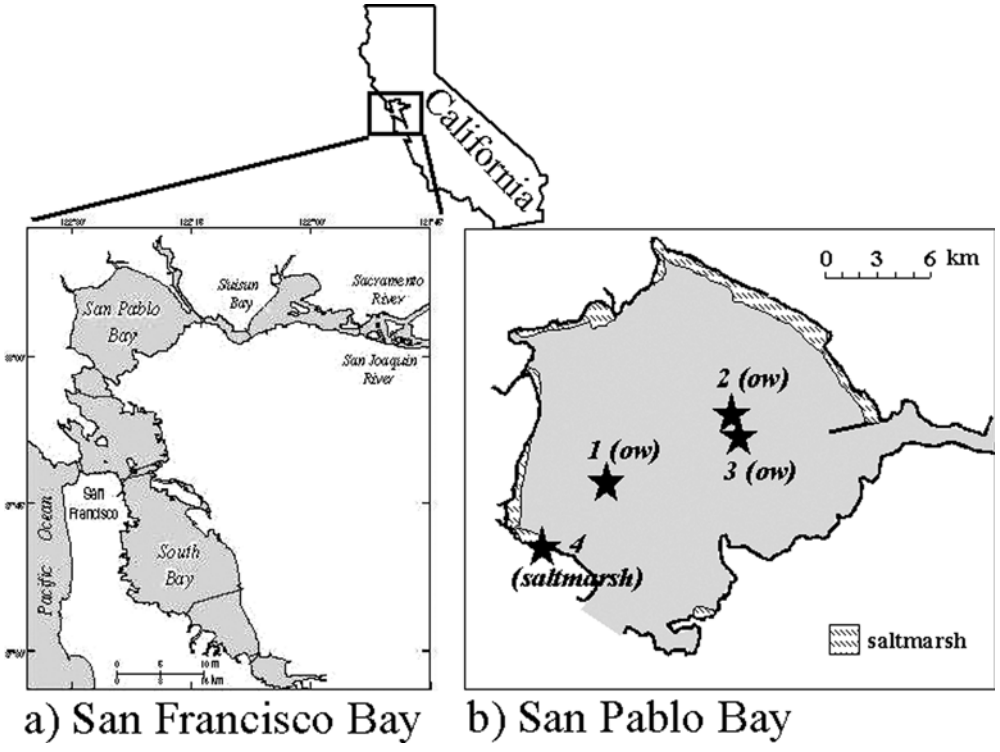

Fig. 1
Site map showing **a** the location of San Pablo Bay within San Francisco Bay and **b** the three open water (ow) sites (nos. 1–3) and the marsh site (no. 4) sampled during March 2000

59002-72). Electrodes were calibrated with commercially available Zobell's solution (Spectrum Quality Products, Gardena CA) or pH buffers (Orion, Beverly MA), respectively, prior to use. Electrodes were directly inserted into sediment (ca. 2 cm into each 4-cm-depth interval) and maintained thus, until a stable reading was achieved. Sediment loss of dry weight upon ignition (%LOI) was measured (APHA 1981) as an index of organic content.

Microbial mercury transformation assays

The initial setup of microbial assays began 1–8 days after the time of original sediment collection. Sediment from a single site/depth was transferred to a clean zip-lock bag and homogenized by physical hand manipulation. Subsamples (3.0±0.1 g) from this composite were transferred to 13-cm^3 serum bottles that were crimp sealed, and the gas phase was flushed with N_2 (O_2-free) to maintain anaerobic conditions. Acid-cleaned stainless steel tools were used for all subsampling. Samples were pre-incubated at room temperature for 2 days, after which, control and $CH_3{}^{203}Hg^+$ extraction efficiency samples (see below) were autoclaved. Each site/depth set consisted of three live samples and one autoclave-killed control for both microbial process measurements (Hg methylation and MeHg degradation).

Mercury methylation rate

The Hg methylation rate was assessed using a $^{203}HgCl_2$ radiolabel approach, a procedure modified from previous studies (Gilmour and Riedel 1995; Stordal and Gill 1995). A working stock solution of $^{203}HgCl_2$ (10 μCi ml^{-1}, specific activity =1.58 μCi μg $Hg(II)^{-1}$, ^{203}Hg half-life =46.5 days) was prepared in phosphate buffer (59 mM KH_2PO_4 final concentration, pH=7.0) from a commercially produced primary stock (Isotope Products Laboratory, Burbank, California). A standard addition of $^{203}HgCl_2$ (100 μl 3 g^{-1} wet sediment) was added to each sample, which resulted in a total Hg(II) amendment of 0.21 μg Hg(II) g^{-1} wet sediment. This was just above or about equal to in-situ total-Hg concentrations (below). Samples were vortexed for 1 min and incubated for 23 h at room temperature. Incubations were arrested by flash freezing samples in a dry-ice/ethanol bath and stored frozen until further analysis. The $CH_3{}^{203}Hg^+$ produced from $^{203}Hg(II)$ was toluene extracted. Thawed sediment was first washed from the serum bottles, into 50-cm^3 fluoropolymer centrifuge tubes, with the following succession of reagents: 4 m urea (4 ml), 0.5 m $CuSO_4$ (2 ml), 6 m HCl (8 ml), and toluene (10 ml). The centrifuge tube was vortexed for 1 min then placed on a rotating shaker for 15 min. Tubes were then centrifuged (3,000 rpm for 5 min) to separate organic and aqueous phases. The toluene phase was decanted to a second fluoropolymer tube, and another 10 ml addition of toluene was added to the first tube. The vortex, rotation, centrifugation, and decanting steps were then repeated, resulting in a 20-ml combined toluene phase. Anhydrous Na_2SO_4 was added (ca. 0.5 g) to the toluene fraction to adsorb any trace amounts of water, which may have contained unmethylated $^{203}Hg(II)$. The toluene was subsampled (15 ml) and counted for radioactivity on an EG and G Wallace Gamma Counter (model 1480) operated in cpm mode. First-order rate constants for Hg methylation (k_{meth}) were calculated as $k_{meth}=\ln(1-f_m)/t$, where f_m equals the fraction $^{203}Hg(II)$ converted to $CH_3{}^{203}Hg$ and t equals the incubation time.

Duplicate subsamples, prepared as above, were collected from each site/depth to measure the efficiency of the above for MeHg extraction procedure. Autoclave killed samples were amended with ^{14}C-MeHg (as below) and subsequently subject to the same toluene extraction as were the ^{203}Hg amended samples. Recoveries of ^{14}C-MeHg ranged from 74–84% for all sites and depths. The appropriate MeHg-efficiency correction was applied to all ^{203}Hg methylation rate calculations.

Methylmercury degradation rate

Microbial MeHg degradation was measured in a set of similarly prepared samples, which were incubated concurrently with the ^{203}Hg methylation set. These samples were amended with $^{14}CH_3HgI$ (10 nCi 100 μl^{-1}, specific activity =54 nCi nmol, ^{14}C half-life =5,730 years, Amersham Pharmacia Biotech, Helsinki, Finland) which was equivalent to 12 ng g^{-1} wet sediment (as Hg). These levels were 5- to 230-fold greater than in-situ MeHg concentrations (below). Incubations were arrested with the addition of 3 N NaOH (1 ml), and assayed for gaseous degradation end products ($^{14}CH_4$ and $^{14}CO_2$) with a previously described $^{14}CH_4$ combustion and $^{14}CO_2$ trapping method (Marvin-DiPasquale and Oremland 1998). First-order rate constants for MeHg-degradation (k_{deg}) were calculated as $k_{deg}=\ln(1-f_d)/t$, where f_d equals the fraction ^{14}C-MeHg converted to total gaseous end-products ($^{14}CH_4$ plus $^{14}CO_2$) and t again equals the incubation time.

Mercury speciation

Sediment total mercury (Hg_t) was quantified using sediment acid digestion, stannous chloride reduction of Hg(II) to Hg^0, gold trapping and cold vapor atomic fluorescence spectrophotometry (CVAFS) detection of Hg^0 (Gill and Fitzgerald 1987; Bloom and Fitzgerald 1988). Sediment MeHg was assayed by acid chloride distillation (Horvat and others 1993), aqueous-phase ethylation, G-C separation and CVAFS detection (Bloom 1989). Quality control consisted of duplicate measurements per site/depth, standards prepared from commercial reagents [$HgCl_{2\ (aq)}$ and $CH_3HgCl_{\ (solid)}$], matrix-spike additions, and the analysis of certified reference material (CRM) and reagent blanks. All samples were assayed in duplicate on two separate occasions because of the less than optimal matrix spike recoveries (for Hg_T) and CRM verification results (for MeHg) obtained during the first analysis. Quality control results are reported for both assaying events. Absolute detection limits were 0.02–0.25 ng (mean=0.09, n=4) and 0.02–0.06 ng (n=2) for the Hg_t and MeHg analysis, respectively. The relative standard difference of duplicate measurements ranged from 0–27% (mean=8.8%, n=16) for Hg_t and 4–76% (mean=28%, n=16) for MeHg. Matrix spike recoveries were 74±14% (n=5) for Hg_t (as $HgCl_2$) and 86±3% (n=3) for MeHg (as CH_3HgCl). CRM consisted of marine sediment (PACS-II,

3.04±0.20 μg g^{-1} dry wt., National Research Council Canada) in the case of Hg_t and dogfish muscle (DORM-II, 4.47±0.35 μg g^{-1} dry wt., National Research Council Canada) in the case of MeHg. Measured CRM concentrations were 108±19% (n=2) and 76±16% (n=3) of the mean certified values, respectively. The Hg_t and MeHg concentrations reported below were not corrected for these matrix spike or CRM results.

Assessment of hydraulic mining debris geochemical signature

Neodymium (Nd) isotopes are powerful indicators of sediment provenance (DePaolo 1988; Nelson and DePaolo 1988; Linn and others 1992) and have been successfully used in the San Francisco Bay estuary to identify zones of historic mining debris deposition in sediments (Bouse and others 1996). Nd isotopic ratios were assayed on the <64-μm fraction of dry bulk sediment (200 mg samples), which was initially digested in HF (29 N, 2 ml) and HNO_3 (16 N, 0.5 ml) for 2 weeks at 100 °C in fluoropolymer beakers. The samples were taken to dryness, HNO_3 (16 N, 2.0 ml) was again added, and samples were held at 100 °C for another 48 h. The samples were again taken to dryness, then digested overnight in HCl (6 N, 5 ml). After another drying step, several drops of H_2O_2 (30%) were added and the samples were redried. Finally, HCl (2 N, 2 ml) was added, and the samples were heated for several hours and then cooled. The Nd analyte was separated first on a 200–400 mesh Dowex (AG-50WX8) resin, using 2 and 6 N HCl as an eluent, to remover rare earth elements. The analyte was then eluted on a 200–400 mesh ammoniated Dowex (AG-50WX4) resin, using 0.15 and 0.225 N methyl lactic acid as the eluent. Isotopic composition was quantified with a Finnigan Mat 261 thermal ionization mass spectrometer, using a Nd standard ($^{143}Nd/^{144}Nd$=0.512140± 0.00002; NASA-Ames). Isotopic ratios were normalized to correct for mass fractionation such that $^{146}Nd/^{144}Nd$=0.72190. The sediment isotopic deviation from the traditional chondritic uniform reservoir (CHUR) standard ($^{143}Nd/^{144}Nd_{CHUR}$=0.512636) was then calculated as (DePaolo and Wasserburg 1976):

$$\varepsilon\mathrm{Nd} = \left(\frac{\left({}^{143}\mathrm{Nd}/{}^{144}\mathrm{Nd}_{\mathrm{measured}} - 1 \right)}{\left({}^{143}\mathrm{Nd}/{}^{144}\mathrm{Nd}_{\mathrm{CHUR}} \right)} \right) \times 10,000 \qquad (1)$$

Results and discussion

The two approaches used to distinguish the presence of hydraulic mining waste in San Pablo Bay were total Hg concentrations and Nd isotopic signature. Previous research in the Bay has demonstrated that sediment deposited during the time of hydraulic gold-mining has lower ϵNd values (–5.0 to –7.0) than sediment deposited before hydraulic mining began (–2.7 to –3.7; Bouse and others 1996). By comparison, sediment ϵNd values from five areas of large-scale hydraulic gold-mining in the Yuba, Bear, and American River watersheds ranged between –5.6 and –13.9. For the current study, ϵNd values (Table 1) were similar in the surface sediment (0–4 cm) of all the open water sites (range: –3.76 to –3.92). These values were only slightly higher than background (pre-mining era) ϵNd, suggesting the presence of a small amount of mining debris. The narrow range of values in these surface sediments suggests that the three sites are similar and well mixed. This conclusion is supported by the Hg_T data (Fig. 2), which also exhibited little among-site variation. Below the 0–4-cm interval, in site 3, there is a decrease in ϵNd (range: –4.28 to –4.35), and increase in Hg_T (Fig. 3), suggesting a higher contribution of mining debris at depth. However, the ϵNd values are well within the range seen in other SFB cores for sediment deposited after 1952 (age determined by ^{137}Cs; Fuller and others 1999). The marsh site exhibited the highest ϵNd (–3.08), which was comparable to pre-mining era background levels. This result was unexpected because this marsh area was originally created during the hydraulic mining era, resulting from the massive sediment input to northern SFB during this period. The background level ϵNd value indicates that the original sediment load that created this marsh is buried at some depth below the shallow surface interval sampled. This is reasonable in light of the high burial rates typical in productive organic-rich marsh areas in general.

The reconstructed bathymetry model predicted that the hydraulic mining debris layer should be at the sediment

Table 1
Isotopic $^{143}Nd/^{144}Nd$ ratios and calculated ϵNd. Data for San Pablo Bay (CA) sediment collected during March 2000. The standard deviations of n=3 measurements from a single sediment sample are given in parentheses for the ratio data

Site	Depth interval (cm)	$^{143}Nd/^{144}Nd$		ϵNd
1 (ow)	0–4	0.512439	(0.000009)	–3.84
2 (ow)	0–4	0.512443	(0.000005)	–3.76
3 (ow)	0–4	0.512432	(0.000011)	–3.98
4 (marsh)	0–4	0.512478	(0.000014)	–3.08
3 (ow)	0–4	0.512432	(0.000011)	–3.98
3 (ow)	8–12	0.512414	(0.000008)	–4.33
3 (ow)	16–20	0.512412	(0.000008)	–4.37
3 (ow)[a]	22–26	0.512417	(0.000025)	–4.28

[a]Averaged data from two separate sediment samples

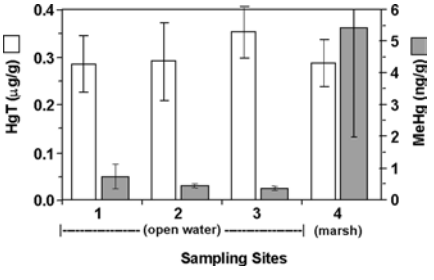

Fig. 2
Total mercury (Hg_t) and methylmercury (MeHg) concentrations in San Pablo Bay (CA) 0–4 cm surface sediment from all four sampling sites. *Error bars* equal one standard deviation of the mean of duplicate samples measured twice (i.e., n=4). Note different scales

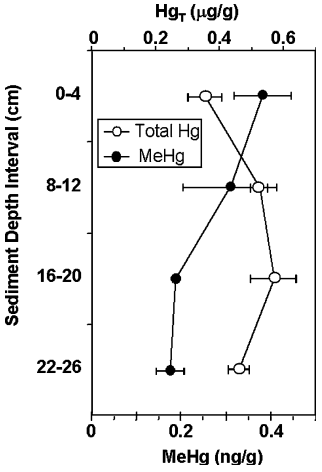

Fig. 3
Sediment depth profiles of mercury (Hg_t) and methylmercury (MeHg) concentration in San Pablo Bay (CA) at site 3. *Error bars* equal one standard deviation of the mean of duplicate samples measured twice (i.e., n=4). Note different scales

surface at site 2 and either absent or some distance below the surface at sites 1 and 3, respectively. However, both Hg_T and Nd results suggest a similar level of historic Hg contamination at all open-water sites. The biotic and abiotic mixing of surface sediment, in this shallow high-energy environment (Fuller and others 1999), undoubtedly dilute Hg-enriched mining debris with less contaminated sediment as the hydraulic mining horizon becomes re-exposed in regions of net sediment loss (e.g., site 2). Similar small among-site variation in surface sediment concentrations has been observed for other pollutants in the San Pablo Bay (Ritson and others 1999). Whereas model predicted among-site differences were not observed for the 0–4-cm horizon in this study, other verification studies indicate that the model is accurate in predicting the sediment depth with geochemical characteristics of mining debris (Bouse et al, in preparation). Indeed, the model did correctly predict the increase in Hg_t with depth at site 3 (Fig. 3), although, deep profiles for sites 1 and 2 do not exist for comparison. The total Hg_t concentrations measured at all sites and depths were below the USEPA criteria for contaminated sediment (>1.0 ppm; Nichols and others 1991), but were similar to previously measured values in San Pablo Bay (Hornberger and others 1999) and other SFB locations (Bouse and others 1996; SFEI 1999). The spatial trends in MeHg concentration did not parallel those for Hg_t. MeHg in the surface (0–4 cm) sediment was low (0.45–0.75 ppb) in all open-water sites compared with 5.4±3.5 ppb in the marsh (Figs. 2 and 3). There was an overall decrease with sediment depth, from 0.38 to 0.17 ppb MeHg, at site 3 (Fig. 3). Values of k_{meth} (Table 2) were below the detection limit (<0.0003 day^{-1}) in all open-water (sites 1–3) surface sediments (0–4 cm), and highest (0.014 day^{-1}) at the marsh (site 4). The comparatively high MeHg concentration in the presence of low k_{meth}, observed in the 0–4 cm horizon at site 3 (Fig. 3, Table 2), indicates rapid recycling of a large pool of MeHg in this surface layer. For short incubation times (hours), the ^{203}Hg methylation measurement may approximate a gross rate measurement (Gilmour and Riedel 1995; Krabbenhoft and others 1997). However, because the incubation time in the current study was comparatively long (24 h), the ^{203}Hg methylation rate measurement may tend towards a net value, particularly if gross MeHg degradation was rapid, as observed (see below). Subsequently, a minimal net ^{203}Hg methylation potential would be expected. Alternatively, the trend in MeHg concentration and k_{meth} at site 3 may reflect the upward diffusion of MeHg produced in reducing sediment below 0–4 cm, followed by the effective binding of MeHg onto Fe and Mn oxides in the comparatively oxidized surface layer (Gagnon and others 1996; Gill and others 1999). The redox profile for site 3 supports this hypothesis (Table 3), as do earlier reports of net MeHg production being greatest in anoxic sediments (Porvari and Verta 1995).

MeHg production was highest for the marsh, even though this sediment was the most oxidized (Table 3). This initially appears to counter the reasoning above for site 3. The oxidized conditions observed in freshly collected marsh sediment likely result from the extensive pickleweed roots associated with this sample, which facilitate O_2 transport below the sediment surface. Although sulfate-reducing bacteria (SRB) are strict anaerobes, their activity is often enhanced in root zones compared with

Table 2
^{203}Hg methylation rate constants (k_{meth}), potential rates, and in-situ rates. Data for San Pablo Bay (CA) sediment collected during March 2000. Standard deviations (n=3) are given in parentheses

Site	Depth interval (cm)	k_{meth} (day^{-1})		Potential Hg methylation rate[a] (ng g^{-1} wet sediment day^{-1})		In-situ Hg methylation rate[b] (ng g^{-1} wet sediment day^{-1})	
1 (ow)	0–4[c]	<3.0E-04		<0.06		<0.04	
2 (ow)	0–4[c]	<3.0E-04		<0.06		<0.03	
3 (ow)	0–4[c]	<3.0E-04		<0.06		<0.04	
4 (marsh)	0–4[c]	1.4E-02	(6.6E-03)	3.10	(1.42)	1.78	(0.88)
3 (ow)	0–4[c]	<3.0E-04		<0.06		<0.04	
3 (ow)	8–12	4.9E-03	(8.2E-04)	1.05	(0.18)	1.04	(0.18)
3 (ow)	16–20	2.3E-03	(4.5E-04)	0.50	(0.10)	0.53	(0.12)
3 (ow)	22–26	1.0E-03	(5.8E-04)	0.22	(0.12)	0.20	(0.11)

[a]Calculated as k_{meth} multiplied by the total Hg(II) amendment (0.21 μg g^{-1} wet sediment)
[b]Calculated as k_{meth} multiplied by the total inorganic Hg(II) concentration (as Hg_t – MeHg)
[c]Below k_{meth} detection limit of 3.0E-04 day^{-1}

Table 3
Sediment redox (Eh), pH, and dry weight loss on ignition (LOI). Data for San Pablo Bay (CA) sediment collected during March 2000. The deviation of duplicate (n=2) measurements are given in parentheses where appropriate. If no such value appears, only a single measure was made

Site	Depth interval (cm)	E_h (mV)[a]		pH		% LOI	
1 (ow)	0–4	+131	(38)	7.5	(0.1)	6.2	
2 (ow)	0–4	+139	(29)	7.6		6.7	(0.1)
3 (ow)	0–4	+193		7.4		6.8	(0.1)
4 (marsh)	0–4	+421	(5)	5.8	(0.1)	12.9	(0.5)
3 (ow)	0–4	+193		7.4		6.8	(0.1)
3 (ow)	8–12	+23		7.8		6.6	(0.1)
3 (ow)	16–20	–20		8.0		7.0	(0.3)
3 (ow)	22–26	–24		8.0		6.8	(0.3)

[a]$E_h=E_m+E_r$, Where E_m = the measured value (in mV) taken with the platinum electrode and E_r is the temperature dependent correction for a standard hydrogen reference electrode (+207 to +211 mV)

non-vegetated sites (Isaksen and Finster 1996; Blaabjerg and others 1998). These bacteria may readily exist in "oxidized" sediments by taking advantage of reduced micro-zones within the substrate (Jørgensen 1977). In addition, SRB are the primary methylators of inorganic Hg(II) in anoxic estuarine sediments (Compeau and Bartha 1985). Because all incubations were carried out under anoxic conditions, the activity of resident SRB may have increased in the marsh samples relative to their original activity under more oxidizing conditions. This, combined with the higher organic content of the marsh sediment (LOI =12.9%) relative to the open-water sites (LOI=6.2–7.0%), may have stimulated SRB, thereby contributing to the observed high Hg methylation rates at this site. Marsh sediment was also considerably more acidic (pH=5.8) than all open-water sites (pH=7.4–8.0; Table 3). This low pH presumably results from high organic acid concentrations, which are generated from the fermentative degradation of this organic-rich substrate. It has been shown in freshwater systems that MeHg production is enhanced under low pH condition (Winfrey and Rudd 1990). Thus, pH may also partially account for the higher k_{meth} values and MeHg concentrations in the marsh.

The spatial trend for microbial MeHg degradation paralleled that of MeHg production, as k_{deg} values were similar (0.15–0.25 day^{-1}) at all open-water sites (0–4 cm) and highest for the marsh (Table 4). Degradation rates decreased tenfold from 0–4 to 22–26 cm at site 3. These k_{deg}'s were tenfold faster than the k_{meth} values, suggesting that the ^{14}C-MeHg was more readily available to microbes than was the ^{203}Hg(II) for methylation. Similar results have been observed in samples from the Florida Everglades (Marvin-DiPasquale and Oremland 1998) and the heavily Hg-contaminated Carson River Superfund site (M. Marvin-DiPasquale, unpublished results). The ^{14}C-MeHg degradation measurement is undoubtedly a gross measurement as essentially none of the gaseous end-product ($^{14}CH_4$ or $^{14}CO_2$) is likely to be re-assimilated into ^{14}C-MeHg. Thus, these results suggest the rapid recycling of MeHg in the surface sediment of site 3, as net (presumed) MeHg production was low and gross MeHg degradation was high.

$^{14}CO_2$ comprised 61–95% of the total ^{14}C-end-products ($^{14}CO_2$ plus $^{14}CH_4$), in ^{14}C-MeHg degradation experiments, at all sites and depths (data not shown). Similar high $^{14}CO_2$ ratios have been observed in a variety of other systems, including SFB (Marvin-DiPasquale and Oremland 1998; Oremland and others 1991, 1995). This is thought to reflect the degradation of MeHg by an oxidative pathway as opposed to the inducible *mer*-detoxification pathway (Marvin-DiPasquale and others 2000). These results indicate that oxidative demethylation is a primary pathway for MeHg degradation, even in sediments elevated in Hg as a result of historic mining debris. Because there is no known Hg(II)-reduction to volatile Hg^0 step associated with OD, the implications for San Pablo Bay sediments is that the inorganic Hg(II), liberated as a result of MeHg decomposition, presumably has a longer residence time

Table 4
^{14}C-MeHg-degradation rate constants (k_{deg}), potential rates, and in-situ rates. Data for San Pablo Bay (CA) sediment collected during March 2000. Standard deviations (n=3) are given in parentheses

Site	Depth interval (cm)	k_{deg}[a] (day^{-1})		Potential MeHg degradation rate[a] (ng g^{-1} wet sediment day^{-1})		In-situ MeHg degradation rate[b] (ng g^{-1} wet sediment day^{-1})	
1 (ow)	0–4	0.150	(0.049)	1.87	(0.62)	0.043	(0.027)
2 (ow)	0–4	0.221	(0.007)	2.77	(0.09)	0.035	(0.005)
3 (ow)	0–4	0.183	(0.004)	2.28	(0.05)	0.025	(0.004)
4 (marsh)	0–4	0.251	(0.079)	3.14	(0.98)	0.532	(0.378)
3 (ow)	0–4	0.183	(0.004)	2.28	(0.05)	0.025	(0.004)
3 (ow)	8–12	0.038	(0.007)	0.47	(0.08)	0.005	(0.002)
3 (ow)	16–20	0.033	(0.018)	0.41	(0.22)	0.002	(0.001)
3 (ow)	22–26	0.019	(0.002)	0.24	(0.02)	0.001	(0.000)

[a]Detection limit =0.002 day^{-1}
[a]Calculated as k_{deg} multiplied by the total MeHg amendment (12.5 ng g^{-1} wet sediment, as Hg)
[b]Calculated as k_{deg} multiplied by the total in-situ MeHg concentration

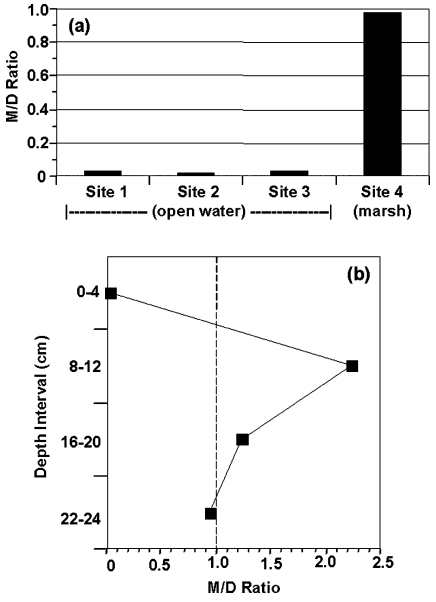

Fig. 4
Mercury methylation/degradation (M/D) ratios of potential rate data given in Tables 2 and 4 in San Pablo Bay (CA) for **a** 0–4-cm surface sediment from all four sites and **b** the down-core profile from site 3

compared with if the primary pathway was *mer*-detoxification. Thus, MeHg degraded via OD may be rapidly remethylated, and the Hg component cycled many times between organic and inorganic forms until it is ultimately take up by the food web or buried.

MeHg production and degradation potential rates are calculated as the k_{meth} and k_{deg} values multiplied by the total Hg(II) and MeHg amendments, respectively (Tables 2 and 4). Similarly, apparent in-situ rates are calculated as the product of the appropriate rate constants and the in-situ Hg-species concentration. The resulting potential and in-situ rates are similar for Hg methylation because the ^{203}HgCl amendment (0.21 μg g wet sed^{-1}) was similar to the in-situ Hg(II) concentration. In contrast, MeHg degradation potential rates are much higher than in-situ rates because the total MeHg in the ^{14}C-MeHg amendment was 5–230× higher than in-situ MeHg levels.

Direct comparisons of calculated methylation and demethylation rates must be approached with caution. The in-situ rate calculation would imply that all of the in-situ inorganic mercury (Hg_t minus MeHg) is available for methylation, and that all of the MeHg is available for degradation. However, this is likely not the case. It is currently uncertain exactly what physically associated fraction, speciated form, and percentage of in-situ Hg(II) (and MeHg) is available to bacteria. To simplify matters, it is assumed that the bioavailability of the Hg-radiotracers to bacteria approximates that for in-situ Hg-pools, and that the measured k_{meth} and k_{deg} values reflect the transformation rates of the microbially available Hg-pools only. The methylation/demethylation (M/D) ratio calculated from the potential rates than gives us some index of the relative transformation rates for bioavailable Hg(II) and MeHg pools. If k_{meth} values reflect gross methylation, than M/D values above unity would indicate net methylation, and values less than unity would indicate net MeHg degradation. However, any such assumptions about k_{meth} reflecting strictly gross methylation may be erroneous (as discussed above). Nonetheless, M/D ratios do provide us with a comparative index for these two competing processes. The resulting spatial trends (Fig. 4) support the conclusion that net Hg methylation is greatest in the marsh site and just below the oxidized surface sediment in the open-water site 3.

Conclusion

Results from this preliminary study confirm earlier findings that surface sediments of San Pablo Bay are enriched in total mercury as a result of historic hydraulic mining practices. Although Hg_t concentrations were very similar in surface sediments at all sites, strong differences in MeHg concentrations and microbial Hg-transformation rates among-sites suggests that sediment geochemistry (redox, sulfide, pH, organic content, etc.) is a much more important control on MeHg production than is the absolute Hg_t concentration. As a result, marsh sediments located around the periphery of the bay appear to be the most active zones for net MeHg production, presumably because of both the organic-rich nature of these zones, and the ability of rooted macrophytes to supply molecular oxygen at depth in the sediment, thus keeping reduced-S levels low. This has important implications for the wetland reconstruction proposed for this area. Without sufficient safeguards, substantial wetland reclamation efforts may result in an overall increase in MeHg production in these newly constructed wetlands, with net MeHg export to the larger bay. Because only one wetland site was sampled in this study, more work is clearly needed to verify the overall contribution of various wetland habitats to total MeHg production in the SFB estuary.

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