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# A Quantitative Relationship that Demonstrates Mercury Methylation Rates in Marine Sediments Are Based on the Community Composition and Activity of Sulfate-Reducing Bacteria

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A quantitative framework was developed which estimates mercury methylation rates (MMR) in sediment cores based on measured sulfate reduction rates (SRR) and the community composition sulfate-reducing bacterial consortia. MMR and SRR as well as group-specific 16S rRNA concentrations (as quantified by probe signal) associated with sulfate-reducing bacteria (SRB) were measured in triplicate cores of saltmarsh sediments. Utilizing previously documented conversion factors in conjunction with field observations of sulfate reduction, MMR were calculated, and the results were compared to experimentally derived measurements of MMR. Using our novel field data collected in saltmarsh sediment where sulfate reduction activity is high, calculated and independently measured MMR results were consistently within an order of magnitude and displayed similar trends with sediment depth. In an estuarine sediment where sulfate reduction activity was low, calculated and observed MMR diverged by greater than an order of magnitude, but again trends with depth were similar. We have expanded the small database generated to date on mercury methylation in sulfur-rich marine sediments. The quantitative framework we have developed further elucidates the coupling of mercury methylation to sulfate reduction by basing calculated rates of mercury methylation on the activity and community composition of sulfate-reducing bacteria. The quantitative framework may also provide a promising alternative to the difficult and hazardous determination of MMR using radiolabeled mercury.

## Introduction

The accumulation of methylmercury in aquatic systems continues to pose a threat to fish and other biota including man. Methylmercury is the most toxic form of mercury (Hg) which is particularly susceptible to biomagnification in aquatic food webs (*1*). In previous studies of sediments and bacterial pure cultures, sulfate-reducing bacteria (SRB) have been implicated as the primary biological mediators of methymercury production (*2*−*9*). Using molybdate, a specific metabolic inhibitor of sulfate reduction, previous research has demonstrated that sulfate reduction is coupled to Hg methylation in aquatic sediments (*3, 6, 10*). Pure cultures of SRB grown in the presence/absence of sulfate were shown to couple sulfate respiration to Hg methylation (*11, 12*).

The saltmarsh is an optimal environment in which to model the coupling of Hg methylation to sulfate reduction. Sulfate reduction has been shown to dominate the biogeochemistry of saltmarsh sediments (*13, 14*), and the SRB as mentioned above are very abundant in these environments (*14, 15*). Furthermore, Hg methylation, even in highly sulfidic portions of saltmarsh sediment, has been shown to be tightly coupled to sulfate reduction and sulfate-reducing bacteria (*10, 11, 16*).

Recent studies conducted in marine sediments have determined the quantitative relationship between SRR and MMR (*10*). With the advent of genetic methods, the population composition of SRB has been elucidated in marine sediments using molecular probes that target the 16S rRNA gene (*4, 11, 14, 17, 18*). These studies have revealed that the SRB are an important bacterial population in saltmarsh sediments, comprising up to 43% of the rRNA from members of the domain bacteria and present at $10^6-10^7$ cells per gram sediment (*14*). Further, the *Desulfobacteraceae*, an SRB family capable of complete acetate oxidation, was observed to dominate over other families of the SRB population in the saltmarsh (*11, 14*). Despite the recent advances in community composition and the activities of the SRB in marine sediments, few studies have combined the approaches (*4, 11*).

Recently, King et al. (*11*) observed that different phylogenetic groups of SRB methylate mercury at different rates when MMR was normalized to the SRR on a per cell basis in pure cultures and marine sediments. Moreover, members of the *Desulfobacteraceae* family were observed to produce methylmercury at more rapid rates than the *Desulfovibrionaceae* when MMR was normalized to SRR (*11*). Because of substantial differences in the electron donors utlilized by these SRB families, it was hypothesized that mercury methylation is coupled to carbon metabolism in SRB.

A rate function ($f^*$) was then developed as a means of quantifying MMR when normalized to the SRR (*11*). Since SRB in marine sediments have demonstrated a tight coupling between MMR and SRR, the $f^*$ term provides a means of quantitatively relating methylmercury production to sulfate reduction rates in sediments. In pure culture, the $f^*$ term is denoted as $f_i^*$ to identify the uniqueness of rate functions among phylogenetic groups of SRB (*11*).

The most accurate MMR measurements to date have been provided by adding radiolabeled $^{203}$Hg to sediment cores or slurries and counting the amount of radioactive methylmercury formed overtime using a chemical extraction method (*8*). The use of radiolabled Hg has several drawbacks including cost and custom synthesis of $^{203}$Hg, utilization of a gamma-emitting radioisotope, and radioactive hazardous waste production from extractions. Thus, the advent of a quantitative framework based on less costly and more environmentally friendly measurements would aid in studies of the fate of toxic methylmercury in sediments.

Since the advent of oligonucleotide probes that target the 16S rRNA molecule of SRB in freshwater and marine systems,

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several researchers have used the technology to characterize the community composition and function of SRB populations in various sediments (*4, 11, 12, 14, 18*). In this study, we utilize profiles of SRB distributions based on group-specific quantification of the 16S rRNA, SRR profiles estimated from $^{35}SO_4^{2-}$ whole core incubations, and pure culture measurements of $f_i$* to interpret measurements of MMR in saltmarsh sediments. Additionally, we demonstrate the use of a quantitative framework that provides an extension of the pure culture derived $f_i$* term while taking into account the sediment SRR and SRB profiles to calculate a MMR.

## Experimental Section

**Sediment Sampling.** Sediment cores (10 cm) were collected at low tide in a saltmarsh along the Skidaway River adjacent to the Skidaway Institute of Oceanography in Savannah, GA. Intact cores were used which consisted of a plexi-glass cylinder (12 cm long and 1.90 cm diameter) with a Teflon plunger. Incubations of sediment cores were initiated within 1 h of retrieval. Intact cores were sealed immediately after sampling to maintain anoxic conditions during incubation. Injection ports in the core barrels were placed 1 cm apart for the entire length of the core barrel and then sealed with silicon sealant. Intact cores were equilibrated and incubated at the in situ temperature (27 °C) and sectioned after incubation into 1 cm intervals for analysis. One, 3, 6, and 3 replicate cores were used for sulfate, SRR, MMR, and 16S rRNA, respectively. Cores utilized for MMR analysis were also used to determine the dry weight of sediment.

**Analytical Methods.** SRR was determined using the radiotracer technique developed by Jørgensen (*19*) with a one-step sulfide distillation performed according to Fossing and Jørgensen (*20*). SRR determinations were performed by injection of 1 $\mu$L of 1 $\mu$Ci/$\mu$L radioactive $^{35}SO_4^{2-}$ (2.2 mCi-mL$^{-1}$; ICN Biomedical, Inc.) into intact sediment cores at 1 cm intervals. The core was allowed to incubate for 6 h at 27 °C after which it was sectioned into 20 mL of 20% zinc acetate and analyzed as described below. Sulfate concentrations were determined using the turbidometric method of Tabatabi (*21*).

For MMR incubations, an injection of 2 $\mu$L (0.5 $\mu$g/$\mu$L) mercuric nitrate was made at 1 cm intervals. Intact cores analyzed for methylmercury were sampled immediately after mercury addition (i.e., 0 h) and 12 h after Hg addition. The 12 h time period was chosen based on results published by King et al. (*10*). After incubation, the MMR cores were sliced at 1 cm intervals and stored at −80 °C until analysis. MMR calculations were based on the amount of methylmercury produced over the 12 h time period. $CH_3Hg$ concentrations were determined using methods reported in King et al. (*10*). Briefly, $CH_3Hg$ was quantified using CVAFS as in Liang et al. (*22*) and modified by King et al. (*10*).

**Use of Oligonucleotide Probes.** For 16S rRNA determination in sample cores, cores were collected and sectioned at 2 cm intervals then frozen at −80 °C until analysis. Methods used for extraction and purification of microbial 16S rRNA from sediments were based on those reported by Moran et al. (*12*). Extracted nucleic acids were hybridized to oligonucleotide probes specific to major SRB groups (Table 1) using a slot blotting procedure (*23*). Purified rRNA from axenic cultures of SRB (*12*) was used to calibrate the probes. SRB cultures were maintained and grown as previously described (*11*). Oligonucleotide probes were synthesized at the Molecular Genetics Facility of the University of Georgia using an ABI DNA/RNA synthesizer (model 394). The labeling of oligonucleotides and probing of 16S rRNA was carried out as in Devereux et al. (*23*). The appropriate [$^{32}$P] labeled probe was added to the blots followed by the addition of 15 mL of fresh prehybridization solution that had been warmed to 55 °C. Hybridization was allowed to occur over 18 h in a shaking circulating water bath at 55 °C. Blots for each probe were washed at 55 °C three times (20 min each wash) in deep dishes that contained 50 mL of wash buffer. After final washes, blots were exposed to film (Fuji Medical X-ray film, 20.3 × 25.4 cm, Fuji Medical Systems U.S.A.) in autoradiograph cassettes (FBXC 810, Fisher Scientific) with enhancement screen at −80 °C. Film was taken out of cassettes and developed based on methods of Frischer et al. (*24*). The specificity of probes and quantification of 16S rRNA was determined using a densitometer (420 OE, PDI Inc.).

**TABLE 1. $f_i$* Term for Pure Cultures of Sulfate-Reducing Bacteria (SRB)**

| SRB | $f_i$* term ± % SE (nmol/nmol) | SRR ± % SE (nmol-cell$^{-1}$ h$^{-1}$) |
|---|---|---|
| DSV *Desulfovibrio desulfuricans* Probe 687 | $1.4 \times 10^{-6} \pm 12.8$ | $1.4 \times 10^{-7} \pm 7.9$ |
| DSB *Desulfobulbus propionicus* Probe 660 | $2.9 \times 10^{-7} \pm 34.1$ | $7.0 \times 10^{-8} \pm 14.6$ |
| DSC *Desulfococcus multivorans* Probe 814 | $4.6 \times 10^{-6} \pm 28.2$ | $2.7 \times 10^{-7} \pm 20.4$ |
| DBACTER *Desulfobacter sp.* BG-8 Probe 129 | $4.1 \times 10^{-6} \pm 23.4$ | $1.4 \times 10^{-6} \pm 13.7$ |
| DSBM *Desulfobacterium* sp. BG-33 Probe 221 | $2.58 \times 10^{-5} \pm 11.1$ | $1.1 \times 10^{-6} \pm 8.7$ |

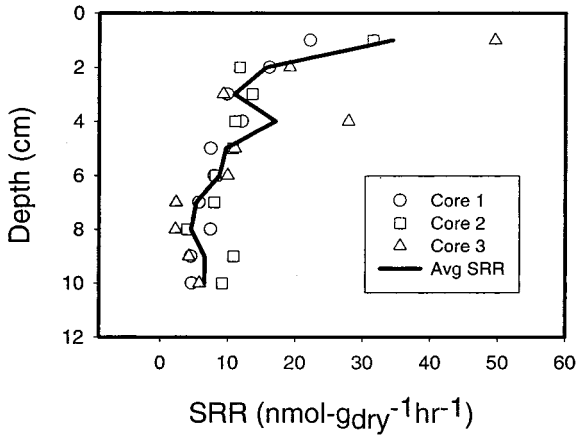


**FIGURE 1. Sulfate-reduction rates (SRR) measured in triplicate sediment cores collected in a saltmarsh adjacent to the Skidaway River, Savannah, GA.**

## Field Results

Sulfate-reduction rates (SRR) in triplicate sediment cores displayed a maximum in surface sediments (0−2 cm depth) and decreased with sediment depth (Figure 1). The highest variability in SRR was observed in the 1-cm depth interval where values ranged from 22.15 to 49.6 nmol/$g_{dry}$-h (Figure 1). In general, SRR values decreased to < 13.0 nmol/$g_{dry}$-h at depths greater than 3 cm (Figure 1).

Directly measured mercury methylation rates MMR showed a similar trend to SRR with increasing depth in the same sediments and ranged on average from 119.2 pg/$g_{dry}$-h to 7.7 pg/$g_{dry}$-h (Figure 2). The data indicate that maximum mercury methylation rates were located in the upper 4 cm of all cores (Figure 2). Both SRR and MMR showed relatively consistent, lower rates in the deeper sediments sampled (7−10 cm depth).

In triplicate cores sampled from the same saltmarsh site as for microbial rate measurements, the composition of SRB

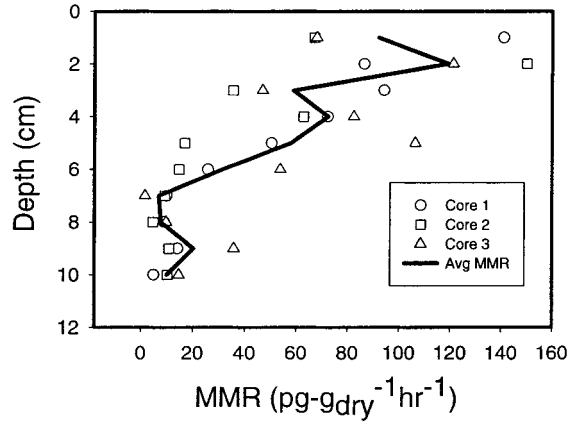


**FIGURE 2. Mercury methylation rates (MMR) observed in triplicate sediment cores collected at the Skidaway saltmarsh.**

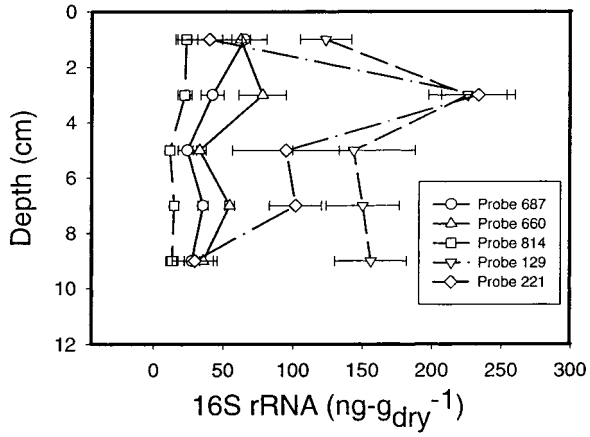


**FIGURE 3. Community composition of SRB analyzed in triplicate sediment cores from the Skidaway saltmarsh. 16S rRNA concentrations were quantified from probe signal as described in text.**

consortia was quantified using group-specific oligonucleotide probes that targeted the 16S rRNA molecule. *Desulfobacterium* and *Desulfobacter* groups were shown to be the most abundant SRB present as indicated by the highest 16S rRNA concentrations (quantified by probe signal), and a subsurface maximum was observed at approximately 3 cm depth (Figure 3). *Desulfovibrio* and *Desulfobulbus* groups were detected at intermediate 16S rRNA concentrations and decreased slightly with sediment depth. The *Desulfococcus* group was observed in the lowest concentration and did not change with sediment depth.

## Discussion and Development of a Quantitative Framework

Previous studies using pure cultures of SRB indicated that MMR for different phylogenetic groups varied over 3 orders of magnitude when normalized to the SRR (*11*). Moreover, these results also suggested that the ability to utilize acetate as a carbon source is a significant factor in determining the incidence of methylmercury production among the phylogenetic groups. Previous work also identified *Desulfobacteriaceae* as the most abundant SRB family and related MMR to this group in saltmarsh sediments (*25*). This was demonstrated in the reported MMR for the genera *Desulfobacterium* and *Desulfobacter* which are capable of complete acetate oxidation (*11*, *26*).

Field data collected at the same site as the King et al. study (*10*) was used in conjunction with $f_i$* data derived from pure culture studies to predict MMR in sediment cores taken from the Skidaway River marsh (Table 1).

Equation 1 defines the MMR normalized to the SRR as the *net* incidence term, *f**.

$$\frac{\mathrm{MMR}}{\mathrm{SSR}} = \hat{f}\left[\frac{[\mathrm{Hg}]}{K_{[\mathrm{Hg}]} + [\mathrm{Hg}]}\right] = f^* \qquad (1)$$

Since the methylation of mercury is coupled to sulfate reduction and activity may vary between different groups, the rate of respiration by individual groups must be taken into account. The *net* rate function includes both a rate function ($\hat{f}$) and a Hg bioavailability term. In subsequent equations the *f** value is expressed as $f_i$* in order to designate the rate function contribution for each phylogenetic group of SRB (*11*). The metabolic activity is potentially different for each phylogenetic group, and thus the separate contribution of each group is considered in the equation. The fact that the SRR varied among individual phylogenetic groups in pure culture suggested that the calculated SRR in the natural sediments cannot be equally allocated to the individual SRB phylogenetic groups. Moreover, this consideration provides each SRB phylogenetic group with a different unit activity in terms of methylmercury production since the SRR is a component that determines MMR.

Equation 2 describes the MMR as the sum of respective rates for each individual phylogenetic group.

$$\mathrm{MMR}_{\mathrm{Total}} = \Sigma\mathrm{MMR}_i \qquad (2)$$

From earlier equations it can be restated that the individual $\mathrm{MMR}_i$ is a function of the $\hat{f}_i$ term as well as the individual SRR and available mercury (eq 3).

$$\mathrm{MMR}_i = \hat{f}_i\mathrm{SRR}_i\left[\frac{[\mathrm{Hg}]}{K_{i[\mathrm{Hg}]} + [\mathrm{Hg}]}\right] = f_i^*\mathrm{SRR}_i \qquad (3)$$

In Equation 3, $\hat{f}_i$ defined the maximum quantity of methylmercury produced per sulfate reduced by a specified phylogenetic group. The $K_{i[\mathrm{Hg}]}$ term represents a half-saturation constant for cellular internalization/transport of mercury (i.e. mercury availability) for methylation. The $f_i$* term groups the $\hat{f}_i$ term and mercury availability term together. The individual $\mathrm{SRR}_i$ contribution of each phylogenetic group present in the sediment cores was not known. Thus, the observed SRR of a sediment depth would be a function of individual phylogenetic groups present at that depth and the unit SRR activity of that group. The fraction of a total group present ($16S\ \mathrm{rRNA}_i/16S\ \mathrm{rRNA}_{tot}$) represents a method to account for specific phylogenetic groups that compose a fraction of the total consortium with respect to depth. The unit activity of the groups can be expressed in terms of the SRR observed in pure culture normalized to cell number ($\mathrm{SRR}_{pc}/\mathrm{Cell}_{pc}$). The $\mathrm{SRR}_i$ can now be evaluated based on the observed SRR in the sediment ($\mathrm{SRR}_{total}$), fraction of 16S rRNA for each individual group ($16S\ \mathrm{rRNA}_i$), and the fraction of SRR unit activity of each group expressed in terms of the pure culture SRR ($\mathrm{SRR}_{pc}/\mathrm{Cell}_{pc}$).

Equation 4 illustrates the calculation of the individual contributions of $\mathrm{SRR}_i$.

$$\mathrm{SRR}_i = \mathrm{SRR}_{\mathrm{total}}\left[\frac{16S\ \mathrm{rRNA}_i \cdot \left(\frac{\mathrm{SRR}_{pc}}{\mathrm{Cell}_{pc}}\right)_i}{\Sigma\left(16S\ \mathrm{rRNA}_i \cdot \left(\frac{\mathrm{SRR}_{pc}}{\mathrm{Cell}_{pc}}\right)_i\right)}\right] \qquad (4)$$

Table 2 indicates the sum total amount of 16S rRNA ($\mathrm{ng/g_{dry}}$) for all five SRB phylogenetic groups present at each depth as well as the percentage of 16S rRNA of each group at that depth in saltmarsh sediments.

**TABLE 2. Percentage of 16S rRNA as Determined by Oligonucleotide Probes in Sediment Cores**

| depth interval (cm) | sum of total SRB16S rRNA ($ng/g_{dry}$) | percentage 16s rRNA detected with Probe 687 | Probe 660 | Probe 814 | Probe 129 | Probe 221 |
|---|---|---|---|---|---|---|
| 0−2 | 314.1 | 20.7 | 19.9 | 7.4 | 39.3 | 12.7 |
| 2−4 | 601.0 | 7.0 | 13.0 | 3.7 | 37.5 | 38.8 |
| 4−6 | 306.4 | 7.8 | 10.7 | 3.7 | 46.9 | 30.9 |
| 6−8 | 356.4 | 9.9 | 15.3 | 4.1 | 42.1 | 28.6 |
| 8−10 | 261.4 | 10.5 | 13.6 | 5.0 | 59.6 | 11.3 |

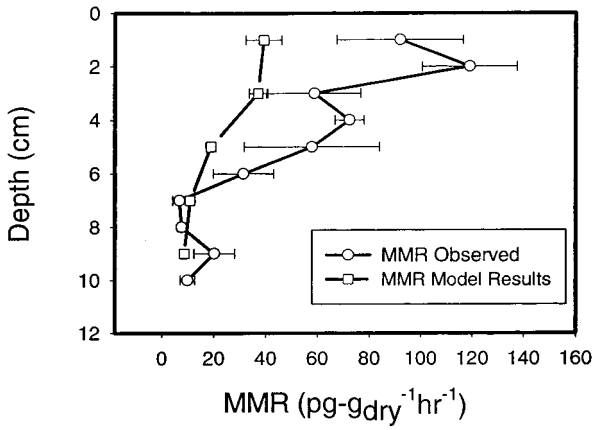


**FIGURE 4. Comparison of observed MMR and MMR predicted from eq 6. Observed MMR were determined from the accumulation of methylmercury in intact cores taken from the Skidaway marsh.**

Substituting eq 4 into eq 3 results in an expression (eq 5) that allows for the calculation of $MMR_i$ as function of $f_i$*, the SRR observed with respect to depth in the core, the fraction of a specific phylogenetic group that is present at a depth, and the SRR unit activity for each phylogenetic group.

$$MMR_i = f_i^* \, SRR_{total} \left[ \frac{16S \text{ rRNA}_i \cdot \left( \frac{SRR_{pc}}{Cell_{pc}} \right)_i}{\Sigma \left( 16S \text{ rRNA}_i \cdot \left( \frac{SRR_{pc}}{Cell_{pc}} \right)_i \right)} \right] \quad (5)$$

Equation 2 defined sediment core MMR as the sum total of individual MMR (i.e., $MMR_i$) contributions from the various SRB phylogenetic groups found within the core. Thus, the $MMR_i$ term in eq 5 can be substituted into eq 2 resulting in a final equation (eq 6) that allows for the calculation of MMR in sediments.

$$MMR_i = \Sigma f_i^* \, SRR_{total} \left[ \frac{16S \text{ rRNA}_i \cdot \left( \frac{SRR_{pc}}{Cell_{pc}} \right)_i}{\Sigma \left( 16S \text{ rRNA}_i \cdot \left( \frac{SRR_{pc}}{Cell_{pc}} \right)_i \right)} \right] \quad (6)$$

Since the $f_i$* term is not known for the phylogenetic groups in the sediment cores, $f_i$* terms (nmol methylmercury produced/nmol sulfate reduced; Table 1) defined in pure culture experiments will be substituted into eq 6 (*11*). The $f_i$* term is reported in Table 1.

Using our field data from the Skidaway marsh, calculated MMR are compared to the observed MMR for the same sediment (Figure 4). Overall, observed, and predicted MMR show a direct correlation and compare to within an order of magnitude (Figure 4). Calculated MMR values indicate that maximum MMR exist in the 2−4 cm region (Figure 4). This is followed by a decrease in calculated MMR as depth increases. The MMR that were observed in sediment cores support this general trend and suggest that maximum mercury methylation rates in the range of 140−80 $pg/g_{dry}$-h typically occur at a depth of 2−5 cm (Figure 4). This is followed by a sustained decrease in MMR (generally 10−15 $pg/g_{dry}$-h) at depths greater than 6 cm (Figure 4).

Figure 4 clearly indicates that discrepancies exist between actual MMR values and calculated MMR values. Several studies have demonstrated that methylation activity in marine sediments is limited by the bioavailibility of mercury (*10, 11, 27−29*). It should be noted that we have measured "potential" rates of MMR using inorganic mercury that was injected into intact cores. A large concentration of inorganic mercury added directly to sediment cores could represent a significantly larger and more bioavailable pool of mercury in comparison to the mercury available in contaminated sediments. Thus, more inorganic mercury would be available for methylation and this could explain the higher observed MMR when compared to the calculated MMR values (Figure 4). To test for the impact of added Hg on the comparison of the calculated vs experimentally measured MMR, it would be helpful to test the quantitative framework on sediments where MMR has been measured with the radioactive tracer method.

Another limitation of the quantitative framework is the use of pure culture data ($f_i$*) to predict rates of field processes. Equation 3 demonstrates how the $f_i$* term is dependent upon the amount of mercury available for methylation. Certainly, the speciation/availability of mercury in pure culture is different than mercury injected into a sediment column. These differences were so far not taken into account in the quantitative framework and probably contribute to discrepancies between observed and calculated MMR. As mentioned previously, this quantitative framework emphasizes that SRB distributions and respiration activity are critical parameters for the elucidation of calculated MMR. The incorporation of a third parameter that considers mercury bioavailablity would be a significant contribution to the quantitative framework.

The biological and chemical mechanisms controlling Hg speciation in estuarine sediments have been reviewed by several authors (*3, 6, 30*). Methyl Hg production in estuarine sediments is thought to be mainly coupled to biotic sulfate reduction catalyzed by sulfate-reducing bacteria (*11, 31*). Methylation has also been linked to a range of chemical variables including the following: salinity, pH, alkalinity, sulfate, dissolved sulfide, DOC, and bulk phase organic C concentration in sediments (*6*). Barkay et al. (*27*) have suggested that increases in salinity may cause a decrease in the bioavailability of Hg(II) for methylation due to charge differences. At high dissolved sulfide concentrations, the precipitation of HgS is thought to prevent methylation (*9*), but this notion should be more rigorously tested since methylation has been observed in saltmarsh sediments which are naturally high in organic carbon and free sulfides (*10, 16, 31, 32*).

Though less information is available, demethylation of monomethyl Hg has been demonstrated in sediments at rates similar to methylation (*6, 15*). To date, sulfate-reducing and methanogenic bacteria are thought to dominate the degradation of $CH_3Hg$ at in situ Hg concentrations via the oxidative demethylation (OD) pathway in anoxic sediments, and the defining characteristic appears to be oxidation of the $CH_3$ group to $CO_2$, either with or without concurrent $CH_4$ production (*33, 34*). Elemental Hg has been suggested as the major product of demethylation under aerobic or anaerobic conditions, and the production of $Hg^0$ from Hg(II) has been cited as a major sink for Hg from aquatic systems (*35*). However, it has yet to be determined whether Hg(II) reduction to $Hg^0$ occurs in conjunction with OD in estuarine sediments

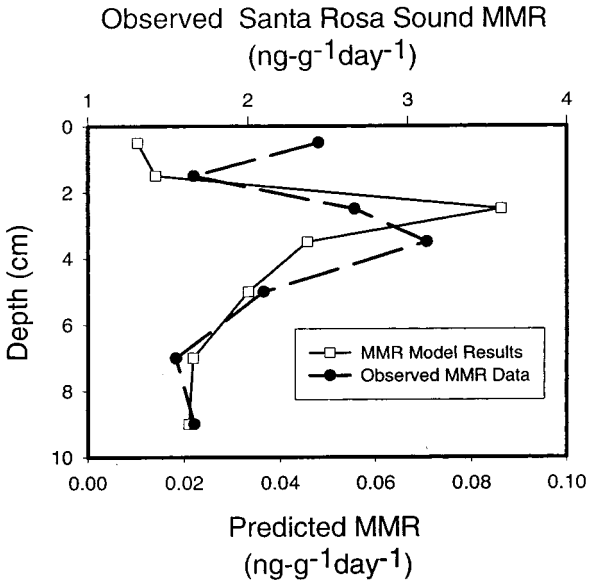


**FIGURE 5. Comparison of MMR and MMR predicted from eq 6. Observed MMR were determined by Deveruex et al. (1996) using $Hg^{203}$ in salt marsh sediments taken from Santa Rosa Sound, FL.**

since liberated Hg(II) may be readily remethylated or react with reduced S species in the absence of significant $Hg^0$ production.

In general, though master chemical variables have been identified and mechanisms have been suggested for the controls of Hg speciation/bioavailability in sediments (*6*), a large amount of uncertainty remains on the actual controls of speciation under real sediment conditions, especially in the coastal marine environment. A bioavailability term for mercury was not input directly into our quantitative framework. Rather, it was integrated into the *f** term from data collected in pure cultures (*11*). Based on the discussion above, knowledge of mercury speciation as well as sediment processes influencing bioavailability would provide expanded range and accuracy for the quantitative framework if incorporated into the calculations. Nonetheless, results presented in this publication illustrate that SRB respiration (i.e., SRR) and the composition of SRB consortia in sediments are major factors affecting the magnitude of methylmercury formation. Additional studies that could provide a mercury availability term for eq 6 would benefit the calculations of MMR tremendously.

To our knowledge, the only other database that relates sediment-based SRB profiles, SRR, and MMR was collected by Devereux et al., (*4*) in Santa Rosa Sound, an estuary in northwest Florida. Sulfate reduction rates were not published in the Devereux et al. study, but these were measured and we have obtained the data (Richard Devereux, personal communication) for incorporation into the quantitative framework. Similar to the comparison in the Skidaway marsh, observed and predicted MMR generally displayed similar trends with sediment depth for the study in Santa Rosa Sound (Figure 5). The MMR measured by Devereux et al., however, were on average 40−50 times higher than the MMR predicted from our quantitative framework. Sulfate reduction rates measured in Santa Rosa Sound (0.5−3.5 nmol-$g^{-1}$ $h^{-1}$) were 10 times lower than those measured in the Skidaway saltmarsh (5−35 nmol-$g^{-1}$ $h^{-1}$), yet the MMR observed at Skidaway (10−120 pg-$g^{-1}$-$h^{-1}$) largely overlapped with observed MMR measured using similar techniques in the Santa Rosa Sound (62.5−125 pg-$g^{-1}$-$h^{-1}$). Furthermore, SRR measured in Santa Rosa Sound sediments were well below the average measured in comparable estuarine sediments under similar environmental conditions (*36*). One possible explanation for the lower calculated framework MMR values in our study is the low SRR reported in Santa Rosa Sound. It should be noted that the SRR reported from Santa Rosa Sound were obtained from distillation of the acid-volatile fraction (AVS) of reduced sulfur only and did not include the chromium-reducible fraction. Several studies have concluded that SRR based only on the AVS fraction will underestimate the in situ sulfate reduction rate (*20*, *37*). Without the use of chromium reduction during distillation of radiolabeled sulfur, up to 95% of reduced sulfur (as pyrite and elemental sulfur) may not be accounted for in the rate measurement. Thus, the calculated MMR for Santa Rosa Sound sediments would be significantly lower than the observed MMR because measured SRR for Santa Rosa Sound only reflect a fraction of the sulfate which was actually reduced.

This paper presents a novel field data set on the community structure of SRB and microbial rate measurements (SRR, MMR). With this data set, we have made a first attempt at developing a reliable quantitative framework to determine mercury methylation potential in sediments based on the distribution of SRB, microbial activity, and mercury concentration. In saltmarsh sediments, the quantitative framework predictions fit well with independently measured mercury methylation rates. In the only other sediment for which a suitable database exists (Santa Rosa Sound), the quantitative framework replicated relative rate changes with depth but predicted much lower MMR than those measured. We suggest that the quantitative framework results point to the difficulty of replicating the bioavailability of mercury in sediment incubations such as those used in our observed MMR measurements. Future studies should focus on the identification of mercury species that are bioavailable for methylation. Other areas of consideration should include substantiation of observed differences in MMR when normalized by cell count and SRR as well as validation of phylogenetically grouped rate functions. It is the hope that this quantitative framework will provide a useful tool for the identification of sites at high risk for methylmercury accumulation by predicting MMR based on the distribution and activity of SRB. The quantitative framework may also provide a promising alternative to the laborious and hazardous determination of MMR using radiolabeled Hg.

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## Literature Cited

(1) Mason, R. P.; Laporte, J. M.; Andres, S. *Arch. Environ. Contamination Toxicol.* **2000**, *38*, 283.
(2) Andersson, I.; Parkman, H.; Jernelov, A. *Limnologica.* **1990**, *20*, 347.
(3) Compeau, G.; Bartha, R. *Appl. Environ. Microbiol* **1985**, *50*, 498.
(4) Devereux, R.; Kane, M. D.; Winfrey, J.; Stahl, D. A. *System Appl. Microbiol.* **1992**, *15*, 601.
(5) Gilmour, C. C.; Capone, D. G. *EOS Trans. Am. Geo. Union* **1987**, *68*, 1718.
(6) Gilmour, C. C.; Henry, E. A. *Environ. Pollut.* **1991**, *71*, 131.
(7) Gilmour, C. C.; Henry, E. A.; Mitchell, R. *Environ. Sci. Technol.* **1992**, *26*, 2281.
(8) Gilmour, C. C.; Riedel, G. S. *Water, Air, Soil Pollut.* **1995**, *80*, 747.
(9) Gilmour, C. C.; Riedel, G. S.; Ederington, M. C.; Bell, J. T.; Benoit, J. M.; Gill, G. A.; Stordal, M. C. *Biogeochem.* **1998**, *40*, 327.
(10) King, J. K.; Saunders: F. M.; Lee, R. F.; Jahnke, R. A. *Environ. Tox. Chem.* **1999**, *18*, 1362.
(11) King, J. K.; Kostka, J. E.; Frischer, M. E.; Saunders, F. M. *Appl. Environ. Microbiol.* **2000**, *66*, 2430.

(12) Moran, M. A.; Torsvik, V. L.; Torsvick, T.; Hodson, R. E. *Appl. Environ. Microbiol.* **1993**, *59*, 915.
(13) Fossing, H. In Geochemical Transformations of Sediment Sulfur; Vairavamurthy, M. A., Schoon, M. A. A., Eds.; ACS Symposium Series 612; American Chemical Society: Washington, DC, 1995; pp 348−364.
(14) Hines, M. E.; Evans, R. S.; Sharak-Genthner, B. R.; Willis, S. G.; Friedman, S.; Rooney-Varga, J. N.; Devereux, R. *Appl. Environ. Microbiol.* **1999**, *65*, 2209.
(15) Pak, K. R.; Bartha, R. *Appl. Environ. Microbiol.* **1998**, *64*, 1987.
(16) Kostka, J. E.; Frischer, M. E.; Maruya, K. A.; Cowden, L. *Estuaries* **2001**, submitted for publication.
(17) Rooney-Varga, J. N.; Sharak-Genthner, B. R.; Devereux, R.; Willis, S. G.; Reidman, S. D.; Hines, M. E. *Syst. Appl. Microbiol.* **1998**, *151*, 84.
(18) Trimmer, M.; Purdy, K. J.; Nedwell, D. B. *FEMS Microbiol. Ecol.* **1997**, *24*, 333.
(19) Jørgensen, B. B. *Geomicrobiol. J.* **1978**, *1*, 11.
(20) Fossing, H.; Jørgensen, B. B. *Biogeochem.* **1989**, *8*, 205.
(21) Tabatabi, M. A. *Environ. Lett.* **1974**, *7*, 237.
(22) Liang, L.; Horvat, M.; Bloom, N. S. *Talanta* **1994**, *41*, 371.
(23) Devereux, R.; Kane, M. D.; Winfrey, J.; Stahl, D. A. *System Appl. Microbiol.* **1992**, *15*, 601.
(24) Frischer, M. E.; Floriani, P. J.; Nierzwicki-Bauer, S. A. *Can. J. Microbiol.* **1996**, *42*, 1061.
(25) Rooney-Varga, J. N.; Devereux, R.; Evans, R. S.; Hines, M. E. *Appl. Environ. Microbiol.* **1997**, *63*, 3895.
(26) Purdy, K. J.; Nedwell, D. B.; Embley, T. M.; Akii, S. *FEMS Microbiol. Ecol.* **1997**, *24*, 221.
(27) Barkay, T.; Gillman, M.; Turner, R. R. *Appl. Environ. Microbiol.* **1997**, *63*, 4267.
(28) Benoit, J. M.; Gilmour, C. G.; Mason, R. P.; Heyes, A. *Environ. Sci. Technol.* **1999**, *33*, 951.
(29) Benoit, J. M.; Mason, R. P.; Gilmour, C. C.. *Environ. Tox. Chem.* **1999**, *18*, 2138.
(30) Craig, P. J.; Moreton, P. A. *Water Res.* **1986**, *20*, 1111.
(31) Weber, J. H.; Evans, R.; Jones, S. H.; Hines, M. E. *Chemosphere* **1998**, *36*, 1669.
(32) Bloom, N. S.; Gill, G. A.; Capellino, S.; Dobbs, C.; McShea, L.; Driscoll, C.; Mason, R.; Rudd, J. *Environ. Sci. Technol.* **1999**, *33*, 7.
(33) Marvin-Dipasquale, M.; Agee, J.; McGowen, C.; Oremland, R. S.; Thomas, M.; Krabbenhoft, D.; Gilmour, C. C. *Environ. Sci. Technol.* **2000**, *34*, 4908.
(34) Oremland, R. S.; Miller, L. G.; Dowdle, P.; Connell, T.; Barkay, T. *Appl. Environ. Microbiol.* **1995**, 2745.
(35) Mason, R. P.; Fitzgerald, W. F.; Hurley, J.; Hanson, A. K.; Donaghay, P. L.; Sieburth, J. M. *Limnol. Oceanogr.* **1993**, *38*, 1227.
(36) Jørgensen, B. B. In *Marine Geochemistry*; Schultz, H. D., Zabel, M., Eds.; Springer: Berlin, 2000; Chapter 5.
(37) Howarth, R. W. In *Aquatic Microbiology: An Ecological Approach;* Ford, T. E., Ed.; Blackwell Scientific Publications: Cambridge, MA, 1993; Chapter 10.

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