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Environmental Factors Affecting Mercury Methylation in Estuarine Sediments

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Sulfate-reducing bacteria were found to be the principal mercury methylators in anoxic aquatic sediments (Compeau and Bartha 1985). Nevertheless, a puzzling aspect of the process remained the observed inverse correlation between mercury methylation and sulfate levels (Compeau and Bartha 1987). One would normally expect a positive rather than an inverse correlation, because sulfate reducers require sulfate as an electron acceptor and they compete poorly with the methanogens in its absence (Lovley 1985). As a likely explanation of the paradox, it was pointed out that the H_2S formed in reducing sulfate-rich environments precipitates mercuric ions, rendering them unavailable for methylation (Compeau and Bartha 1983; Berman and Bartha 1986).

In an additional attempt to elucidate the relation of mercury methylation in saltmarsh sediments to sulfate levels and other environmental conditions, we attempted to simplify the situation by removing the effect of H_2S on mercuric ion availability. Prior to the addition of mercuric ions, anaerobic saltmarsh sediments were treated by excess $FeCl_2$, precipitating H_2S as FeS. After this pretreatment, we measured mercury methylation rates and correlated them with the sulfate levels and other parameters of anoxic saltmarsh sediment samples, collected along a land-to-sea transsect.

MATERIALS AND METHODS

During October 1991, duplicate sediment cores were collected along a transsect from the landward to the seaward border of Cheesequake estuary (Fig. 1). The four collection sites were dictated by the prevailing salinities from 7.0 to 20.0‰. A hand corer (Wildco Instruments, Saginaw, MI) equipped with plastic liners was used for taking cores from mudflats exposed at low tide in a manner that preserved their *in situ* state. After retrieval of 5 by 25 cm cores, the two ends of the liner were immediately capped with butyl rubber stoppers. To measure the *in situ* salinity, a salinity probe (Yellow Springs Instrument Co., Yellow Springs, OH) was placed into the hole which was left behind after taking the cores.

Within 2 hr of collection, samples were placed into an anaerobic chamber (PACE 6500; Labline Instruments, Melrose Park, Ill.) in which an atmosphere consisting of 5% H_2, 5% CO_2, 90% N_2 was maintained. For biological analyses, all subsequent manipulations were performed within the anaerobic chamber. Field and laboratory measurements of redox potential (E_h) demonstrated that this

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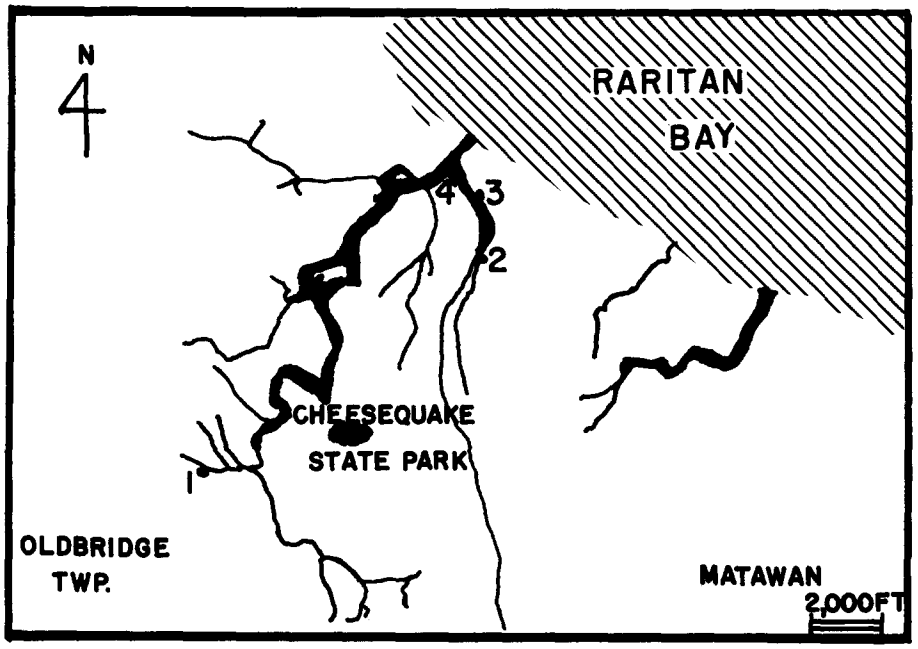

Figure 1. Map of the Cheesequake State Park study area with sampling points (•). Salinities at the each sampling locations were 1 (7 ‰), 2 (12 ‰), 3 (15 ‰), and 4 (20 ‰).

sampling procedure did not change the *in situ* E_h of the sediment.

Total sulfide (free H_2S plus acid-labile sulfides) levels were determined by precipitation with zinc acetate. After placing ~1 g (wet weight) of sediment into a serum vial, sediment sulfide was volatilized by a combination of heat (90°C) and acid (20 mL of 6*N* H_2SO_4) treatment. The volatilized H_2S was flushed out by a gas stream of oxygen-free nitrogen and trapped in two successive vials containing 10 mL of 1*N* zinc acetate each. Subsequently, the ZnS precipitate was collected with a membrane filter (pore size of 0.45 μm, diameter of 25 mm; Whatman Inc., Clifton, N.J.) and iodometric titration was performed according to Howarth (1979). With known concentrations of Na_2S, this method detected sulfide with recovery efficiencies over 92%. The dry weight of the sediment utilized for sulfide determination was subsequently measured and served to calculate mg sulfide per g of dry sediment. The effectiveness of the FeS-precipitation of free H_2S was confirmed by substituting $FeCl_2$ for zinc acetate under the conditions described above. The recovery efficiencies were similar (over 90%) using either a $FeCl_2$ or a zinc acetate trap.

Sulfate concentration in sediment pore water was measured by the gravimetric method described in Standard Methods (1989). Sediment pore water from a known amount of wet sediment was diluted with distilled water and separated by centrifugation. The diluted pore water was then clarified via a membrane filtration (pore size of 0.45 μm, diameter of 47 mm; Whatman Inc.). Subsequently, sulfate was precipitated as $BaSO_4$ by the addition of $BaCl_2$ while boiling the sample at an acidic pH. The solution was kept at 90°C overnight, and the precipitate was collected on an ash-free membrane filter. After an ignition at

800°C for 1 h, the weight of the precipitate was determined, and the sulfate concentration in the pore water was calculated. Data are presented as g sulfate per L of pore water. A recovery efficiency of 95.5 ± 1.4% was obtained with known concentrations of Na_2SO_4.

Sediment organic matter content was measured gravimetrically as weight loss after overnight ignition at 600°C as described in Standard Methods (1989). Units are given as g of organic matter per g of dry sediment.

Mercury methylation and sulfate reduction rates were measured in slurried sediment samples. The water used in making the slurry was deoxygenated artificial seawater prepared with a chemically defined commercial sea salt mix (Seven Seas Marine Mix, Utility Chemical Co., Paterson, N.J.) and diluted to the *in situ* salinity of the sediment. For determination of methylmercury production, 20 mL of slurried sediments received 20 mg of $FeCl_2$ to eliminate free H_2S prior to the addition of 0.5 mg of $HgCl_2$. After incubation at 27°C for 2 days, the slurried sediments were extracted for methylmercury by the method of Longbottom *et al.* (1973). Methylation was stopped by injecting 2.0 ml of a 1.0 M $CuSO_4$ solution into the serum vial containing the sediment slurry. Monomethylmercury levels were measured using a Hewlett-Packard 5890 gas chromatograph equipped with a "macrobore" capillary column (0.53-mm id, 10 m long; Alltech AT-35, Deerfield, Ill.). Operating conditions were as follows: 95:5 Ar-CH_4 (vol/vol) carrier gas (Matheson Gas Products, East Rutherford, N.J.) at 40 ml/min, injector at 210°C, oven at 100°C, and electron capture detector at 250°C. Monomethylmercury peak (retention time of 1.25 min) areas were recorded by a Hewlett-Packard 3392A integrator, calibrated using monomethylmercury standards (American Tokyo Kasei, Inc., Portland, OR) in benzene solution.

Sulfate reduction rates were measured by the conversion of $Na_2{}^{35}SO_4$ (sp act of 794.28 mCi/mmol, Amersham Inc., Arlington Heights, Ill.) to $H_2{}^{35}S$. The produced $H_2{}^{35}S$ was trapped in zinc acetate solution, and collected by membrane filtration as described above. The radioactivity of $Zn^{35}S$ precipitate was counted with a liquid-scintillation counter (BetaTrac 6895; T. M. Analytic, Elk Grove Village, Ill). Counting efficiency was determined by the external standard ratio method.

Correlation analyses were based on simple linear regression, and the calculated correlation coefficients were utilized to assess the relative importance of environmental factors affecting mercury methylation.

RESULTS AND DISCUSSION

Mercury methylation rates, sulfate reduction rates, and various chemical parameters were compared in two sediment depth profiles. In low salinity sediment (7 ‰), both mercury methylation and sulfate reduction rates had their maximum values in the 0 to 4 cm section of the sediment core (Fig. 2). Similarly, sulfate and organic matter content showed maxima near the surface. The same trends were also observed in high-salinity (20 ‰) sediment (data not shown). When these parameters were subjected to correlation analysis, the mercury methylation rate had strong correlations with sulfate reduction rate (r = 0.99), sulfate (r = 0.97), and organic matter content (r = 0.83). A high rate of methylmercury production in the top sediment layer was also observed by other investigators (Jernelov 1970; Berman 1988). In a separate study, it was also

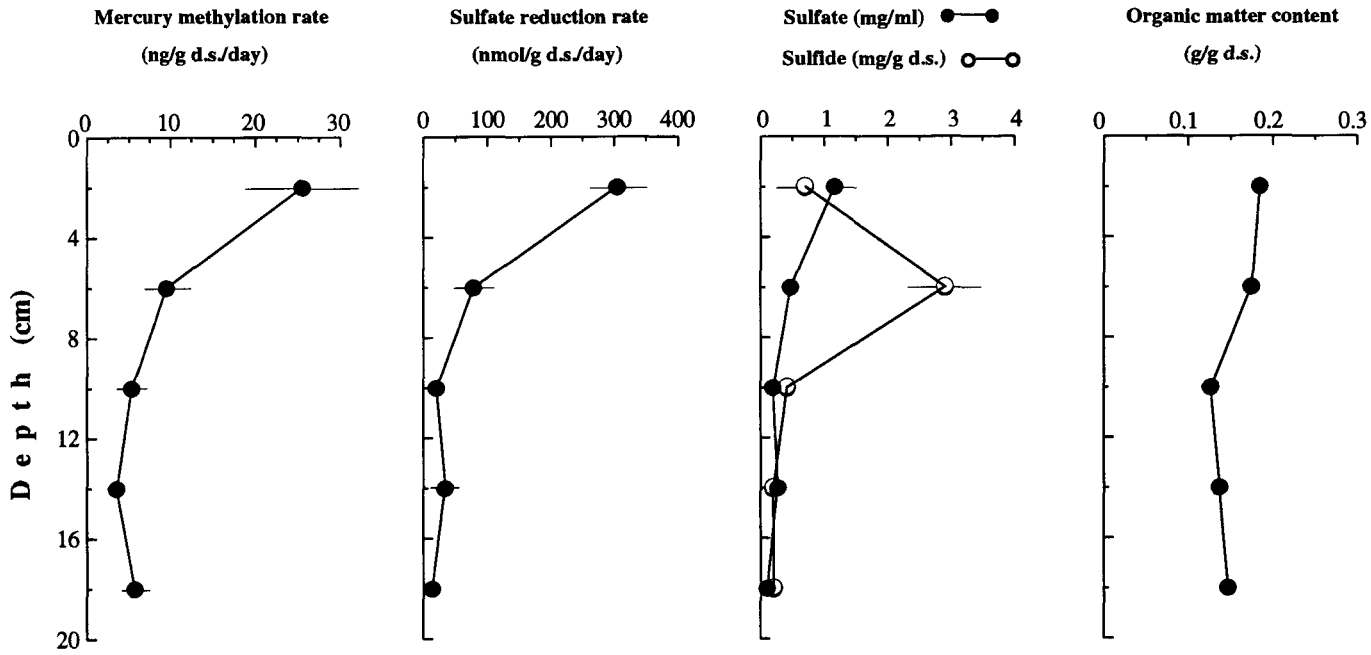

Figure 2. Vertical distribution of mercury methylation rate, sulfate reduction rate, and certain environmental factors in low salinity (7‰) sediment. The high salinity sediment (20‰) had a very similar depth profile (data not shown). The "d.s." abbreviation refers to "dry sediment". The points are the average of triplicate determinations. Bars indicate standard deviation.

found that sulfate reduction occurs most actively within the top layer of the sediment (Jorgensen and Bak 1991). Having compared mercury methylation and sulfate reduction rates in a single sediment core, our results not only confirm these previous findings, but are also consistent with the finding that sulfate reducers are the principal methylators of mercury (Compeau and Bartha 1985).

Sulfide concentrations were not maximal in the uppermost section sediment layer, although sulfate reduction was most active at this depth (Fig. 2). This was probably due to H_2S losses by periodic flushing and aeration with tidal water movement. The surface of the sediment was continuously exposed to air during low tide and, therefore, volatility losses and oxidation could occur. In fact, when the intact sediment core was extruded from the plastic liner, blackening of the sediment by sulfide precipitates was observed only 1 to 2 cm below the surface.

The observed maxima near the sediment surface suggests that the availability of nutrients (organic matter), electron acceptor (sulfate), and the consequent activity of sulfate reducing bacteria are the major controlling factors for methylmercury production within the depth profile of a sediment.

The parameters compared in sediment layers of different depth were also measured in sediments from four locations with different salinities of 7, 12, 15, and 20‰ (Fig. 3). In this case, the most active 0 to 4 cm layers were compared with each other. For sulfate reduction rates, sulfate, and sulfide concentrations, the sites which had salinities of 7 and 20‰ showed higher values than the sites of 12 and 15‰ salinity. The sediment of 15‰ salinity showed particularly low values for all parameters.

The mercury methylation rates, when subjected to correlation analysis, correlated best with sediment organic matter content (Table 1). This is consistent with the often observed increase of methylmercury production with increased levels of organic carbon (Olson and Cooper 1976; Furutani and Rudd 1980; Wright and Hamilton 1982; Lee and Hultberg 1990). However, the previously reported inverse correlation between mercury methylation and salinity (Compeau and Bartha 1987) or sulfide (Craig 1986) was not statistically significant in this study, because any free H_2S was removed in these experiments by precipitating it as FeS. By including this pretreatment, we could measure potential mercury methylation rates which were not limited by the precipitation mercuric ions as HgS.

The mercury methylation rates did not correlate well with sulfate or sulfate reduction rates (Table 1). The likely reason for this is that the range of sulfate concentrations encountered in this transsect were not limiting. It has been generally accepted that 10 mg/l is the sulfate concentration that starts to be limiting for the activities of sulfate reducers (Ingvorsen *et al.* 1981; Lovley and Klug 1983). Even at the low concentrations of sulfate (~ 3 mg/l) encountered in freshwater, sulfate reducing bacteria could remain active by synergistic interspecies H_2 transfer between methanogens and sulfate reducers (Bryant *et al.* 1977; Phelps *et al.* 1985). Among the four samples analyzed in this study, the lowest concentration of sulfate was approximately 500 mg/l which was 50 times higher than the limiting concentration that other investigators have suggested.

Without the H_2S effect on mercuric ion availability and without true sulfate-limitation, sediment organic matter content appears to be the major factor that controls mercury methylation rates in estuarine sediments. This finding adds new

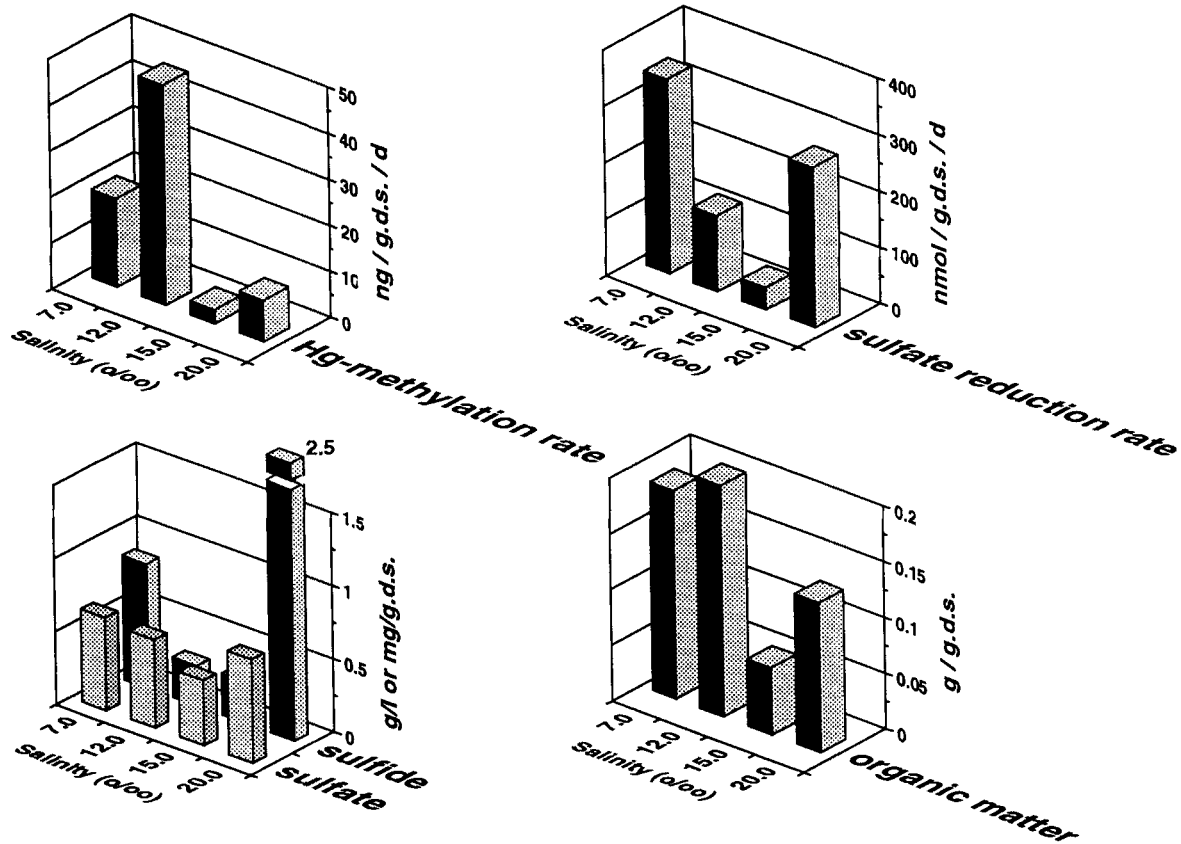

Figure 3. Regional distribution of mercury methylation rates, sulfate reduction rates, and certain environmental factors along a land to sea transsect of Cheesequake estuary.

Table 1. Coefficients of correlation (r) of the factors affecting mercury methylation in locations with different salinities.

Factors	Coefficients of correlation (r)
Sediment organic matter content	0.835
Sulfate concentration	0.234
Sulfate-reduction rate	-0.017
Sulfide concentration	-0.390
Salinity	-0.412

information to previously reported patterns of mercury methylation in anoxic aquatic sediments, but does not contradict the previous publications (Berman and Bartha 1986; Compeau and Bartha 1987)

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