THE ROLE OF MICROORGANISMS IN ELEMENTAL MERCURY FORMATION IN NATURAL WATERS

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Abstract. Gas evasion of elemental Hg (Hg^0) from the open ocean plays a prominent role in the global mercury cycle. Elemental Hg is formed primarily by reduction of ionic Hg in the mixed layer of aquatic systems. By culturing phytoplankton in defined media, and by incubating natural seawater and freshwater samples, we have demonstrated that Hg^0 is produced by microorganisms, with formation rates (0.5 to 10% d^{-1}) similar to those estimated from mass balance studies. Our results also suggest that <3 μm microorganisms are the primary Hg reducers in natural waters. Eucaryotic phytoplankton are capable of reducing ionic Hg to Hg^0 but the rate of reduction is insufficient to account for the observed reduction rates found in incubated field samples. Bacteria are thus the more likely Hg reducers. In seawater, cyanobacteria such as *Synecococcus* may account for much of the mercury reduction, while in the eutrophic, polluted Upper Mystic Lake north of Boston other procaryotic microorganisms are contributing to the overall Hg reductive capacity of the medium. By reducing ionic Hg, microorganisms play a pivotal role in the aquatic biogeochemistry of Hg, not only by enabling evasion to the atmosphere, but by directly decreasing the amount of ionic Hg available for methylation.

1. Introduction

Elemental mercury (Hg^0) plays a fundamental role in the global Hg cycle. The exchange of Hg between surface waters and the atmosphere is rapid as a result of the formation of Hg^0 in natural waters, and its subsequent evasion, (Mason *et al.*, 1994a). In the atmosphere, which is responsible for most of the global Hg transport, about 98% of the Hg is gaseous Hg^0. In the oceans, which are a major source and sink for atmospheric Hg, 10 to 30% of the dissolved Hg is Hg^0 (Kim and Fitzgerald, 1986; Mason and Fitzgerald, 1993); the situation is similar for freshwaters (Vandal *et al.*, 1991; Xiao *et al.*, 1991). As Hg^0 formation removes reactive Hg from the water column where it could otherwise be methylated (Fitzgerald *et al.*, 1994), this process plays an important role in the biogeochemical cycling of Hg in aquatic systems.

However, the mechanisms whereby reactive Hg species are reduced to volatile Hg^0 are poorly known. The reduction appears to be chiefly biological, as suggested by field studies in the equatorial Pacific Ocean (Kim and Fitzgerald, 1986; Mason and Fitzgerald, 1993) and in freshwater and estuarine environments (Kim, 1987; Vandal *et al.*, 1993; Mason *et al.*, 1993a). The inverse correlation between Hg^0 concentration and primary productivity evident from the results of these field studies suggests a casual relationship. There is, however, also evidence for the abiotic reduction of Hg (Alberts *et al.*, 1974; Winfrey and Rudd, 1990; Xiao, 1994). Mass balance calculations suggest

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that reduction rates in the open ocean and in freshwater lakes are in the vicinity of 0.5 to 10 x 10^{-7} s^{-1} (0.5 to 10% d^{-1}; Vandal *et al.*, 1991; Mason *et al.*, 1994b). A recent study in an estuarine mesocosm (Kim, 1987) showed that while there was some relationship between primary productivity and Hg^0 concentration there was no strong association between production of Hg^0 and phytoplankton species composition. The role of bacteria was not investigated.

Studies of Hg reduction by procaryotic microorganisms at high Hg^{2+} concentrations have demonstrated that some bacteria can convert Hg(II) to Hg^0 using a plasmid-encoded enzymatic pathway (the *mer* gene; Belliveau and Trevors, 1989; Robinson and Tuovinen, 1984; Summers and Silver, 1978). Whether this detoxification mechanism is induced at the picomolar Hg concentrations found in natural systems, which are typically 5 to 6 orders of magnitude lower than those used in the laboratory induction experiments, is not known. However, recent studies indicate that microbial reduction of Hg(II) can occur by other mechanisms than the *mer* gene pathway (Oremland *et al.*, 1991; Barkay *et al.*, 1989).

Barkay *et al.* (1989) found that mercury-resistant microorganisms, isolated from lake and estuarine water by culturing at μM Hg concentrations, were able to reduce Hg at rates of 1 to 10% per hour. This study showed that most of the biotic reduction in the isolate from the estuary was due to bacteria (the isolation procedure killed the eucaryotes in the sample) and that other pathways beside the *mer* gene were involved. In contrast, in the freshwater isolate, most of the biotic reduction was due to the *mer* gene pathway. Oremland *et al.* (1991) have shown that demethylation of MeHg, which produces Hg^0, can occur via an oxidative pathway, and that this mechanism could be important in natural waters. Water column demethylation has been demonstrated in freshwater lakes (Xun *et al.*, 1987; Winfrey and Rudd, 1990) although estimated rates of demethylation are less than the estimated rates of Hg^0 formation in the same systems (Vandal *et al.*, 1991; Fitzgerald *et al.*, 1994).

Eucaryotic microorganisms can also reduce Hg (Ben-Bassat and Mayer, 1987, 1988; Bentz, 1977). The algae *Chlorella* reduced Hg in culture when exposed to μM Hg (Ben-Bassat and Mayer, 1977, 1978) although the rate of formation was slow, being on the order of 0.01% of the added Hg per day. These investigators found that the formation of Hg^0 decreased in concert with the inhibition of photosynthesis. Several studies have also shown that phytoplankton can externally reduce Cu(II) and Fe(III) and other metals by cell surface enzymatic processes (Price and Morel, 1990; Jones *et al.*, 1985, 1987). Three reduction pathways were identified for the diatom *Thalassiosira weissflogii*: reduction by organic compounds released into the medium, by cell wall components, and via a plasmalemma enzymatic pathway. Enzymatic reduction was the principal pathway and was inhibited by metabolic inhibitors. Reduction rates were proportional to concentration at low μM concentrations of copper, being about 2% day^{-1} for 10^6 cells/L (1 fmol $cell^{-1}$ hr^{-1} μM^{-1} copper added). We hypothesize that Hg(II) is reduced by algae by a similar mechanism.

Since there is little direct evidence in the literature for biotic reduction of Hg(II) by natural communities of microorganisms exposed to low Hg concentrations, we investigated Hg reduction in laboratory studies with natural samples and phytoplankton cultures. We report here the results of the monoculture experiments and laboratory incubations of field samples which suggest that microorganisms play an

important role in the production of Hg^0 in both freshwater and marine environments.

2. Methods

Cultures were maintained in acid-cleaned polycarbonate bottles, and were spiked from μM $HgCl_2$ standards. Natural samples were collected in acid-cleaned bottles from a depth of about 5 m using a peristaltic pump and acid-cleaned teflon tubing; seawater from the ocean side of Martha's Vineyard, Massachusetts, and freshwater from the Mystic Lakes in Boston (Aurilio *et al.*, 1994). Control (abiotic) samples were either prepared by microwaving the samples until boiling (approx. 6 minutes per liter of sample) or by 0.2 μm filtration. Artificial seawater with added nutrients, trace metals/EDTA and vitamins (Aquil; Price *et al.*, 1989) was used for the culture experiments. As Hg is not significantly complexed by EDTA in the presence of 0.5 M chloride, Hg was added at required concentration, and was not buffered in the solution.

Incubation experiments involved either continuous bubbling of the sample for 3 to 5 hours after Hg addition or 18 to 24 hours batch exposures where samples were not continuously bubbled (Mason *et al.*, 1993b). During initial studies it was found that there could be significant formation of Hg^0 at the start of an experiment. After addition of the Hg spike there was a rapid formation of Hg^0 within the first 20 minutes followed by a slower abiotic production. Other investigators have noted a similar initial production of Hg^0 after addition of Hg (Xiao, 1994) and other trace metals (Jones *et al.*, 1987). This initial abiotic formation, which presumably results from the presence of reductants either in solution or on the container walls, occurs even in distilled water. To remove this initial production of Hg^0, samples were sparged continuously with Hg-free air, and the gaseous Hg was collected periodically after addition. Alternatively, for batch cultures that were not continuously sparged, samples were degassed with air an hour after the addition of the Hg spike to remove any gaseous Hg formed due to the initial reduction. All cultures were maintained under constant fluorescent light (150 μEinstein m^{-2} s^{-1}; Price *et al.*, 1989) at 25°C.

Mercury analyses relied on atomic fluorescence quantification of Hg^0 (Bloom and Fitzgerald, 1988). To determine Hg^0, samples were purged unamended and the volatile Hg trapped on gold columns. For the determination of reactive Hg, tin chloride was used to convert labile Hg species into Hg^0. All sample manipulation and degassing was done under clean room conditions. Details of the analytical techniques are contained in Bloom and Fitzgerald, 1988; Gill and Fitzgerald, 1987; Mason *et al.*, 1993a).

3. Results and Discussion

3.1 ABIOTIC REDUCTION

A number of experiments performed at nM concentrations of Hg showed that abiotic reduction rates in distilled water were higher than in seawater (Table I), as suggested by other investigators (Xiao, 1994; Barkay *et al.*, 1989). Amyot *et al.* (1994) found that light was necessary for Hg^0 formation in an acidic oligotrophic lake.

Addition of hydrogen peroxide, a reducing agent, did not, however, enhance Hg^0 formation suggestive of photosynthetically-mediated Hg^0 production in this lake. Our measured rates in distilled water (1.1% day^{-1}; 1.2 x 10^{-7} s^{-1}) are somewhat lower than those found by others (Alberts *et al.*, 1974; Winfrey and Rudd, 1990). Abiotic reduction rates in artificial seawater were only 40% of the distilled water rates. Further, abiotic formation rates in microwaved lakewater were similarly greater than the rate measured in microwaved seawater (Table I). Because Hg^0 formation rates are also typically lower in seawater (Mason *et al.*, 1994b; Table III) than in freshwater (Vandal *et al.*, 1991, 1993; Table IV) the relative importance of abiotic formation is similar in both systems, from 10 to 30% of the total formation rate.

TABLE I

Abiotic formation rates for elemental Hg in different media under batch culture conditions. Spike concentrations of inorganic Hg were either 0.5 or 0.6 nM.

SAMPLE	Hg^0 (%/day)
DISTILLED WATER	1.1
DISTILLED WATER WITH AQUIL TRACE METALS (ATM)	1.1
MICROWAVED MYSTIC LAKEWATER	1.1 ± 0.6
ARTIFICIAL SEAWATER WITH ATM	0.44
MICROWAVED SEAWATER	0.28 ± 0.2

3.2 MERCURY PRODUCTION BY MARINE MICROORGANISMS

The results of field studies in the equatorial Pacific Ocean (Mason and Fitzgerald, 1993; Mason *et al.*, 1994b) and in the North Atlantic (Mason *et al.*, 1994c) indicated that reduction rates range were between 0.2 and 1% day^{-1} for the open ocean, while higher conversion rates were estimated for the Pettaquamscutt estuary, Rhode Island (Mason *et al.*, 1993a). A number of marine phytoplankton cultures were therefore studied to assess their ability to reduce inorganic mercury to Hg^0 at rates similar to those estimated from field data. Previous studies with *Thalassiosira weissflogii* had shown that this organism could reduce copper at rates of 1 to 10 fmol $cell^{-1}$ hr^{-1} when exposed to 1 to 10 μM Cu(II) (Jones *et al.*, 1987). If reduction rates were similar for Hg, then rates around 0.5 amol $cell^{-1}$ hr^{-1} would be expected at the 0.5 nM exposure concentrations used in the Hg experiments. Our studies with a variety of marine microorganisms showed that the diatom, *T. weissflogii* and the green alga, *Dunaliella tertiolecta* and *Pavlova lutheri*, reduce Hg(II) to Hg^0 at similar rates (0.1 to 0.5 amol $cell^{-1}$ day^{-1}; Table II) while the coccolithophore, *Pleurochrysis caterae*, and the cyanobacterium, *Synecococcus bacillaris*, produced Hg^0 at a slower rate. Preliminary results suggest that *Prochlorococcus*, an abundant open ocean cyanobacteria, also reduced Hg(II) at a rate similar to *Synecococcus*. The formation rates found in these experiments are an order of magnitude lower than those predicted from the experiments with *T. weissflogii* and copper. Because of the low concentration

of Hg relative to other trace metals in the Aquil mixture (Price *et al.*, 1989), competition for reduction sites could account for the slower rates of reduction of Hg, compared to copper, found in the monoculture experiments.

TABLE II

Production of elemental Hg by cultures of marine microorganisms. All production rates are corrected for abiotic production. For the continuous bubbling experiments, abiotic production was 1.3% d^{-1}; for batch exposures, 0.75% d^{-1}. 500 mL samples were spiked to 0.5 nM mercury.

SPECIES	CELLS/L	pg/CELL Chl a$	Hg^0 (%/day)	Hg^0 (amol $cell^{-1}$ d^{-1})	Hg^0 (pmol μg^{-1} Chl a d^{-1})
T.W.	7.7×10^7	1.8	3.0	0.29	0.16
T.W.	7.5×10^6	1.8	0.40	0.48	0.25
D.T.	1.9×10^8	0.5	2.3	0.12	0.25
P.L.	2.4×10^8	0.2	9.7	0.41	2.05
SYN	1.0×10^9	0.01	6.6	0.067	6.6
CCII#	1.3×10^8	0.11	0.35	0.027	0.25

NOTE: T.W. *Thalassiosira weissflogii*, D.T. *Dunaliella tertiolecta*, P.L. *Pavlova lutheri*, SYN *Synecococcus bacillaris*, CCII *Pleurochrysis caterae*.

Batch experiment.

$ Chlorophyll a concentrations per cell were estimated using the general relationship between chlorophyll concentration and cell volume derived by Montagnes et al. (1994).

The production rate of Hg^0 was a function of cell size. *S. bacillaris* is less than 1 μm in radius and its rate of production was highest of the organisms tested on a surface area basis (Mason *et al.*, 1993b) or when normalized to chlorophyll a (Table II). Production rates, in pmol μg^{-1} chl a day^{-1}, were comparable for all the phytoplankton except *Pavlova* and *Synecococcus* which both produced Hg^0 at a higher rate, with the rate for *Synecococcus* being about 30 times greater than the rate for *T. weissflogii*. The cell densities used in these experiments were substantially higher than those found in natural waters, except perhaps for *Synecococcus*. For example, to obtain a conversion rate of 1% day^{-1} with *T. weissflogii* would require a cell density of 10^7 cells/L. At typical oceanic abundances for cells of this size (10^5 cells/L or less) conversion rates would be less than 0.1% d^{-1}; i.e. less than abiotic formation rates. Algae would not be important reducers even at rates of reduction similar to those measured for *P. lutheri*. In contrast, a cyanobacterium such as *Synecococcus*, present at abundances of $> 10^8$ cells/L, could convert Hg at rates comparable to those measured in natural samples (> 0.6% day^{-1} for *Synecococcus*). For the phytoplankton, therefore, it is probable that the smaller cyanobacteria are the important Hg reducers in ocean waters.

A preliminary investigation of Hg^0 production by natural populations of marine microorganisms was conducted using water collected from the ocean off Martha's Vineyard, Massachusetts. Water was pumped into acid-cleaned bottles and transported back to the laboratory for the incubation studies. Subsamples of the water were filtered

and/or amended with additions of trace metals and nutrients and the resultant Hg^0 formation measured (Table III). Distilled water blanks and microwaved samples showed low Hg^0 production (Table I) while the formation rate (corrected for abiotic formation) was 2.3 % day^{-1} for the unamended seawater sample. Addition of trace metals (the normal Aquil mixture; Price *et al.*, 1989) to the culture caused a decrease in the production rate for Hg^0. If Hg and other trace metals are reduced by the same mechanism, then this decrease could be accounted for by the competition between Hg and other reducible trace metals for the available reducing sites.

TABLE III

Formation of elemental mercury in coastal seawater amended with 0.6 nM inorganic Hg and incubated overnight. Formation rates are corrected for abiotic production which was 0.28 $\pm$ 0.19% day^{-1}. The chlorophyll a concentration of the sample was 3 μg/L. Samples were incubated in 500 mL polycarbonate bottles.

SAMPLE	Hg^0 (%/day)	Hg^0 (pmol day^{-1})	Hg^0 (pmol μg^{-1} Chl a day^{-1})
UNFILT.	2.3	6.9	4.6
UNFILT. PLUS ATM*	1.3 $\pm$ 0.6	3.9 $\pm$ 1.8	2.6 $\pm$ 1.2
3 μm FILT.	2.3	6.9	4.6
1 μm FILT	1.1	3.3	2.2

NOTE: * This average includes exposure to three different concentrations of Hg (0.3, 0.6 and 0.9 nM)

Filtration of the sample followed by incubation allowed a preliminary assessment of the size class of the Hg(II) reducing organisms. The >3 μm organisms were clearly not responsible for the reduction (Table III), as suggested by the laboratory culture experiments. The reducing capacity was split between the 1-3 and 0.2-1 μm fractions. The rate of Hg^0 formation for the unamended sample, on a chlorophyll a basis, was similar to that found for *Synecococcus*, suggestive of the role of the cyanobacteria in Hg(II) reduction in ocean waters. Although the data are limited, the results of the laboratory monoculture experiments and the field study indicate that while most marine phytoplankton are capable of reducing Hg(II) to Hg^0, only *Synecococcus* and possibly other cyanobacteria produce Hg^0 at rates comparable to those estimated from field measurement.

3.3 MERCURY REDUCTION IN AN EUTROPHIC LAKE

Elemental Hg formation rates in freshwater lakes have been previously estimated, based on mass balance calculations, to range between 1 and 10% day^{-1} (Vandal *et al.*, 1991; Fitzgerald *et al.*, 1991). Our studies with water collected from the Upper Mystic Lake in Boston over a year and incubated under laboratory conditions, confirm this range in formation rates (Table IV). The Upper Mystic Lake is eutrophic, with blooms of diatoms in the spring and fall and a predominance of cyanobacteria and green algae through the summer (MDC, 1978). The lake has been

historically polluted with high concentrations of arsenic, chromium and other trace metals (Aurilio *et al.*, 1994). Further, there are high concentrations of coliform bacteria (up to 10^5/L) and other heterotrophic bacteria in this lake (Cassidy, 1971). Total Hg concentrations in the Upper Mystic Lake range between 10 and 25 pM with reactive Hg concentrations of 5 pM or less. Elemental Hg concentrations vary throughout the year, ranging from 200 fM to 1.2 pM for the surface waters (Figure 1). Concentrations typically decrease with depth suggesting that the epilimnion is the principal region of Hg^0 formation, as found by Vandal *et al.* (1993). Based on the average reactive Hg concentration (2 pM) and Hg^0 concentration (0.6 pM), conversion rates of 2 to 4% day^{-1} are required to balance the estimated evasional fluxes of 200 to 400 pmol m^{-2} day^{-1}. The Hg^0 formation rates found during the incubation experiments (up to 8% day^{-1}) are generally higher than those estimated for open ocean waters (0.2 to 1% day^{-1}; Mason *et al.*, 1994b; Mason *et al.*, 1994c) but agree with the overall mass balance estimates.

TABLE IV

Formation of elemental Hg in surface water collected throughout the year from the Upper Mystic Lake, Boston. Rates are corrected for abiotic formation. Samples were incubated in 500 mL bottles spiked with 0.5 nM Hg. Chlorophyll a concentrations were measured by HPLC.

DATE	μg/L Chl a	Hg^0 (%/day)	Hg^0 (pmol day	Hg^0 (pmol μg^{-1} Chl a day^{-1})
APRIL 93	7.0*	7.9	20	5.7
MAY	0.35	2.3	5.8	33
JUNE	0.18	0.7	1.8	20
JULY	0.99	5.4; 3.8#	13.5; 9.5	27; 19
SEPTEMBER	0.14	3.4 ± 0.5$	8.5 ± 1.3	120 ± 19
OCTOBER	5.4	5.6	14.0	5.2
NOVEMBER	3.1	<0.5	<1.3	<0.84

NOTE: * The chlorophyll a concentration was determined for this sample by extraction and fluorescence measurement.

\# Samples collected one week apart.

$ Duplicate samples for the same day.

Elemental mercury concentration (Figure 1) and the rate of Hg^0 formation tracked productivity to some extent (Figure 2) with the highest reduction rates in the spring and fall. Both the reduction rate and Hg^0 concentration decreased with depth (Table V and Figure 1). There was also an increase in the Hg^0 reductive ability in July that coincided with an increase in chlorophyll a (Table IV). The correlation between chlorophyll a concentration and Hg^0 formation rate ($r=0.71$) is improved if the anomalous November sample (high chl a, low reduction rate) is ignored ($r=0.88$). Amyot *et al.* (1994) and Vandal *et al.* (1991, 1993) have also found similar relationships between Hg^0 concentration and photosynthetic indicators. While these correlation suggests that there is a link between productivity and reduction, they do not prove that phytoplankton are the primary reducers.

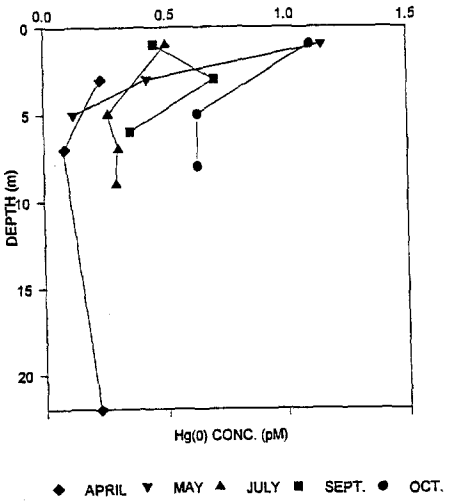

Fig. 1. Measured concentration of elemental Hg in the Upper Mystic Lake over the sampling period (March to November, 1993)

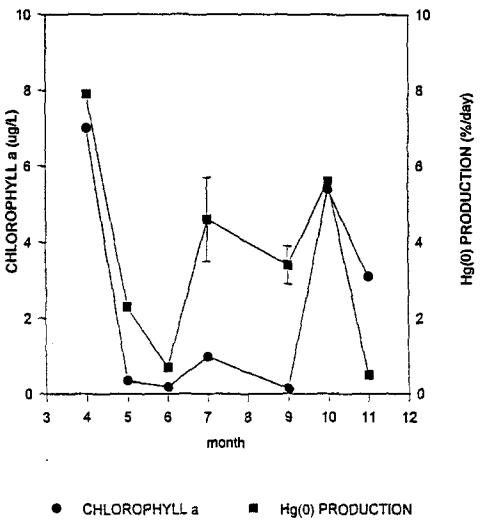

Fig. 2. Variation in chlorophyll a concentration and in the rate of elemental mercury formation for the Upper Mystic Lakes from March to November 1993.

TABLE V
Depth profile for July. Production of elemental Hg with depth in the Upper Mystic Lake. Experimental conditions are the same as in Table IV.

DEPTH (m)	μg/L Chl a	Hg°(%/day)	Hg° (pmol day^{-1})	Hg° (pmol μg^{-1} Chl a day^{-1})
1	0.99	3.8	9.5	19
5	1.75	3.1	7.8	8.9
7	3.36	2.0	5.0	1.3
9	7.19	1.1	2.8	0.8
19	7.84	<0.5	<1.3	0.3

The Hg° production rates, on a pmol μg^{-1} of Chl a basis, are much higher than those measured in the ocean samples or in the culture experiments (Tables II and III). Either the freshwater phytoplankton reduce Hg at a higher rate or other, non-photosynthetic microorganisms are contributing to the Hg° pool. Pigment analysis (Hurley, pers. comm.; Table VI) indicated that the May sample coincided with the end of a bloom (90% of the total chlorophyll a and derivatives was pheophytin a) and that there was little productivity in June. There was an increase in chlorophyll a and fucoxanthin (indicative of diatoms and chrysophytes) in July while the higher chlorophyll a concentrations in October and November coincided with the presence of fucoxanthin and pigments characteristic of cyanobacteria. The Hg° formation rates do not correlate with the presence (October and November) or absence of cyanobacteria. This is also true of a depth profile for Hg° production obtained in July (Table V). Overall, the rate of formation decreased with depth and did not coincide with the increasing chlorophyll a concentration with depth nor with the presence of cyanobacteria in the deepest sample.

Overall, the production rates, on a per chlorophyll basis, vary by at least two orders of magnitude, from <1 to 120 pmol μg^{-1} of chlorophyll a per day. The reduction rates are also, for the most part, higher than those found with the monoculture experiments (<3 pmol μg^{-1} of chlorophyll a day^{-1}) and in the sea water samples (<5 pmol μg^{-1} of chlorophyll a day^{-1}). These results suggest that while chlorophyll a concentrations provide some indication of the reducing ability of the Upper Mystic Lake waters, cyanobacteria and eucaryotic phytoplankton are not the only nor even the principal organisms reducing Hg in this lake.

Size fractionation experiments showed a similar trend to that found in the seawater samples. Most of the reduction was due to less than 3μm organisms (Table VII). In addition, inhibition experiments showed that while inhibition of photosynthesis (addition of DCMU or incubation in the dark) led to 20 to 30% decrease in the reduction rate, addition of the antibiotic ampicillin eliminated most of the reduction, typically an 80 to 90% decrease. These results strongly suggest that bacteria are responsible for most of the Hg° formation in the Upper Mystic Lake. Coupled with the lack of a correlation between Hg° formation and the presence of cyanobacteria, these results suggest that heterotropic microorganisms are the primary reducers in this eutrophic lake and are responsible for the high reduction rates even in the absence of phytoplankton production.

TABLE VI

Concentration of photosynthetic pigments (in nmol per liter) in waters taken from the Upper Mystic Lake. Concentrations determined by HPLC analysis (Hurley pers. comm.).

SAMPLE	fuco	allo	zea	chl b	B-caro	chl a	sum chl a
APRIL						7.7*	
MAY	0	0	0	0	0	0.39	3.97
JUNE	0	0	0	0.07	0	0.20	0.24
JULY	2.25	0	0	0	0	1.10	2.27
SEPT	0	0	0	0	0	0.16	0.33
OCT	0.51	0.33	0.33	0.45	0.86	6.00	7.51
NOV	1.68	0.27	0.27	0.30	0.33	3.47	4.91
JULY 5 m	0.68	0.08	0	0	0	1.92	2.89
JULY 7 m	1.80	0.12	0	0.59	0	3.73	4.81
JULY 9 m	0.45	0	0	0	0	7.98	8.65
JULY 19 m	0.24	0.11	0.26	0	1.23	8.62	8.97

NOTE: * Determined by acetone extraction and fluorescence measurement. Concentration calculated as 1μg/L = 1.1nmol/L for chlorophyll a.

TABLE VII

Production of elemental Hg (% of added Hg reduced per day) by waters collected from the Upper Mystic Lake after filtration or after treatment with inhibitors. Experimental conditions are the same as in Table IV.

TREAT.	4/93	5/93	6/93	7/93	9/93	10/93	11/93
UNFILT.	7.9	2.3	0.7	5.4, 3.8*	3.4$\pm$0.5#	5.6	<0.5
3 μm FILT.	7.0	1.8	1.3			<0.5	
1 μm FILT.	3.9		1.2				
UNFILT +DARK				3.8, 2.6*	2.7		<0.5
+ DCMU				4.3	3.2	3.0	
+ AMPI.				0.7	1.7	0.7	<0.5

NOTES: * Samples collected one week apart
# Duplicate samples from the same day
DCMU = 3-(3,4-dichlorophenyl)-1,1-dimethylurea, an inhibitor of photosynthesis, added at 10μM concentration
AMPI = ampicillin, a broad range antibiotic, added to 50μg/ml concentration
DARK = samples were wrapped in aluminum foil to exclude light

4. Mercury Reduction in Natural Waters

The results of the experiments with natural waters demonstrate that Hg is reduced, primarily by biotic means, at rates comparable to those estimated from mass balance estimates based on field measurements. Further, small organisms (typically < 3 μm in diameter) are the primary Hg reducers. The monoculture experiments and the incubations with seawater and freshwater samples suggest that while eucaryotic phytoplankton are capable of reducing Hg to Hg°, the rate of reduction is insufficient to account for the observed reduction rates in the incubated field samples. Bacteria are thus presumably the more important Hg reducers.

In seawater, the evidence suggests that cyanobacteria such as *Synecococcus* could be important reducers but more studies with specific inhibitors and additional culture experiments are needed to clarify this observation. In addition, the role of heterotrophic bacteria in Hg reduction in seawater has not been studied. Further, it is not known whether the cyanobacteria reduce Hg by a cell surface mechanism or via a gene-encoded pathway. For the eucaryotes, Hg° production could involve cell surface reduction, similar to that found for other trace metals (Jones *et al.*, 1987) rather than a gene coded Hg resistance mechanism.

For the eutrophic Upper Mystic Lake, the results suggest that neither eucaryotic phytoplankton nor cyanobacteria are the main Hg reducers, implying that reduction by heterotophic bacteria is the predominant reduction pathway. A high concentration of coliform bacteria, which are known to reduce Hg at high exposure concentrations (Robinson and Tuovinen, 1984), could account for the difference between the seawater and freshwater samples. The results presented here are consistent with those of Barkay *et al.* (1989). These authors concluded that gene-encoded Hg reduction was the principal biotic reduction mechanism in freshwater, but not in estuarine waters.

In summary, the rate of reduction of Hg in a given aquatic system is a function of the nature of the microorganisms present, particularly the composition of both the autotrophic and heterotrophic procaryotes. Overall this study confirms the Hg° production rate estimates based on field data and has shown that biotic reduction of Hg is the primary route of Hg° formation both in seawater and in freshwaters. Further, this work also provides evidence for the role of procaryotic microorganisms in the reduction of Hg in natural waters.

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References

Alberts, J.J., Schindler, J.E., Miller, R.M, and Nutter, D.E.: 1974, *Science* **184**, 895-897.

Amyot, M., Mierle, G., Lean, D.R.S., and McQueen, D.J.: 1994, Abstract, Conference on *Mercury as a Global Pollutant*, Whistler, BC, July 10-14, 1994.

Aurilio, A.C., Mason, R.P., and Hemond, H.F.: 1994, *Environ. Sci. Technol.*, **28**, 577-585.

Barkay, T., Liebert, C., and Gillman, M.: 1989, *Appl. Environ. Microbiol.* **55**, 1196-1202.

Belliveau, B.H. and Trevors, J.T.: 1989, *Appl. Organometallic Chem.* **3**, 283-294.

Ben-Bassat, D. and Mayer, A.M.: 1977 and 1978, *Physiol. Plant.* **40**, 157-162 and **42**, 33-38.

Bentz, M.: 1977, *Mar. Biol.* **41**, 89-92.

Bloom, N.S. and Fitzgerald, W.F.: 1988, *Anal. Chim. Acta* **208**, 151-161.

Cassidy, R.A.: 1971, The distribution of chromogenic and other heterotrophic bacteria in the Upper Mystic Lake. *M.S. Thesis*, Tufts Univ., Boston.

Fitzgerald, W.F., Vandal, G.M., Mason, R.P. and Dulac, F.: 1994, In: *Mercury as a Global Pollutant: Towards Integration and Synthesis*, (Lewis Publishers), in press.

Gill, G.A. and Fitzgerald, W.F.: 1987, *Global Biogeochem. Cycles*, **3**, 199-212.

Jones, G.J., Waite, T.D. and Smith, J.D.: 1985, *Biochem. Biophys. Res. Comm.*, **128**, 1031-1036.

Jones, G.J., Palenik, B.P. and Morel, F.M.M.: 1987, *J. Phycol* **23**, 237-244.

Kim, J.P.: 1987, Volatilization and Efflux of Mercury from Biologically Productive Ocean Regions, Ph.D. Thesis, University of Connecticut.

Kim, J.P. and Fitzgerald, W.F.: 1986, *Science* **231**, 1131-1133.

Mason, R.P. and Fitzgerald, W.F.: 1993, *Deep-Sea Res.*, **40**, 1897-1924.

Mason, R.P.: 1991, The Chemistry of Mercury in the Equatorial Pacific Ocean, Ph.D., University of Connecticut.

Mason, R.P., Fitzgerald, W.F., Hurley, J.P., Hanson, A.K., Donaghay, P.L. and Sieburth, J.M.: 1993a, *Limnol. Oceanogr.*, **38**, 1227-1241.

Mason, R.P., Morel, F.M.M. and Hemond, H.F.: 1993b, Heavy Metals in the Environment, Nriagu, J.O. and Allan, R.J. (eds.), Vol. 2, CEP Consultants, Edinburgh, pp. 293-296.

Mason, R.P., Fitzgerald, W.F. and Morel, F.M.M.: 1994a, *Geochim. Cosmochim. Acta*, **58**, 3191-3198.

Mason, R.P., O'Donnell, J. and Fitzgerald, W.F.: 1994b, In: *Mercury as a Global Pollutant: Towards Integration and Synthesis* (Lewis Publishers), in press.

Mason, R.P., Rolfhus, K.R. and Fitzgerald, W.F.: 1994c, In this issue of Water, Air, and Soil Pollution.

MDC: 1978, Mystic Rivers Watershed Water Quality Survey, Publ. #11243-49-75-3-79-CR, Commonwealth of Massachusetts.

Montagnes, D.J.S., Berges, J.A., Harrison, P.J., and Taylor, F.J.R.: 1994, *Limnol. Oceanogr.* **39**, 1044-1060.

Oremland, R.S., Culbertson, C.W. and Winfrey, M.R.: 1991, *Appl. Environ. Microbiol.*, **57**, 130-136.

Price, N.M., Harrison, G.L., Hering, J.G., Hudson, R.J., Nirel, P.M.V., Palenik, B. and Morel, F.M.M.: 1989, *Biol. Oceanogr.* **6**, 443-461.

Palenik, B., Kieber, D.J. and Morel, F.M.M.: 1989, *Biol. Oceanogr.* **6**, 347-354.

Price, N.M. and Morel, F.M.M.: 1990, Aquatic Chemical Kinetics (Wiley & Sons, NY) p 235-257.

Robinson, J.B. and Tuovinen, O.H.: 1984, *Microbiol. Rev.*, **48**, 95-124.

Summers, A.O. and Silver, S.: 1978, Annu. Rev. Microbio., **32**, 637-672.

Vandal, G.M., Fitzgerald, W.F. and Mason, R.P.: 1991, *Water Air Soil Poll.* **56**, 791-803.

Vandal, G.M., Fitzgerald, W.F., Lamborg, C.H. and Rolfhus, K.R.: 1993, Heavy Metals in the Environment, Nriagu, J.O. and Allan, R.J. (eds.), Vol. 2, CEP Consultants, Edinburgh, pp. 297-300.

Winfrey, M.R. and Rudd, J.W.M.: 1990, *Environ. Toxicol. Chem.* **6**, 853-869.

Xiao, Z.F.: 1994, In *Mercury as a Global Pollutant: Towards Integration and Synthesis*, Lewis Publishers, (in press).

Xaio, Z.F., Munthe, J., Schroeder, W.H. and Lindqvist, O.: 1991, *Tellus* **41B**, 267-279.

Xun, L., Campbell, N.E.R. and Rudd, J.W.M.: 1987, *Can. J. Fish. Aquat. Sci.*, **44**, 750-75.