Microbial Reduction and Oxidation of Mercury in Freshwater Lakes

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The evasion of elemental mercury represents a significant pathway for reducing the level of this potentially toxic material in aquatic ecosystems. The evasion rate is controlled by the concentration of dissolved gaseous mercury (DGM) across the air−water interface, water, and air temperature as well as wind speed. Here we investigate the role of microbial mercury oxidation and reduction in regulating DGM diel patterns in two freshwater lakes, Jack's Lake and Lake Ontario. Three replicate diurnal cycles of DGM in Brookes Bay, Jack's Lake peaked at 313 fM between 9:00 to 10:30 and decreased to 79.6 fM by 16:00. Microbial mercury reductase activity (converts Hg^{2+} to Hg^0) increased with DGM concentrations and mercury oxidase activity (converts Hg^0 to Hg^{2+}) increased as DGM concentrations decreased in the mid-afternoon. This illustrates that mercury oxidase activity was linked to hydrogen peroxide (H_2O_2) diurnal patterns. Thirty minutes after spiking Lake Ontario water with H_2O_2, mercury oxidase activity increased by 250% and by 60 min, DGM decreased to 28% of its initial value. Two hours after the H_2O_2 spike, mercury oxidase activity had declined, but mercury reductase activity and DGM both increased. Four hours after the spike, mercury reductase and DGM levels had returned to original levels. Our results are consistent with the following sequence of events. In the morning, microbial activity produces DGM (in addition to any DGM formed through photoreduction of Hg^{2+}). As photochemically produced H_2O_2 increases in concentration it induces the biologically mediated decrease in DGM concentrations throughout the afternoon. To predict concentration of DGM in surface waters and flux rates to the atmosphere, the contribution of photoreduction and photooxidation must be placed in context with reduction and oxidation rates due to microbial activity.

Introduction

A significant route of dissolved gaseous mercury (DGM) loss from lakes, rivers, and wetlands occurs by evasion of elemental mercury into the atmosphere. It is a function of the mercury concentration gradient across the water−air interface, the temperature, and the wind speed (*1*−*3*). Strong diel fluctuations in DGM concentrations have been observed in most lakes with levels of DGM peaking at noon (*4*, *5*). Investigators have attributed this increase to photochemical processes that involve the production of reductive species or direct electron transfer (*6*, *7*, *4*). However, neither the reactive species involved nor the mechanism of direct electron transfer initiated by solar radiation has been shown (*8*) although reactive iron is thought to play a role (*9*). The reduction in DGM levels commonly observed in mid-afternoon is thought to be due to increased flux of DGM from lake water as well as mixing of surface water within the epilimnion of lakes (*3*, *10*).

The role of microbial activity in regulating freshwater diel patterns of DGM has been largely ignored. In marine waters, DGM concentrations and phytoplankton pigments are correlated (*11*), but investigators have attributed this correlation to nonspecific reactions occurring as a consequence of microbial growth (*10*). In freshwaters it has been postulated that microbial activity, especially heterotrophic bacteria, may play a role in DGM production in freshwaters (*12*, *13*). These investigators spiked isolated lake water with Hg and observed the production of DGM. However, microbial production of DGM in unpolluted freshwaters has been discounted because mercury concentrations, 3−20 pM, in pristine freshwater environments are below that required for the induction of the bacterial *mer* operon, 50 pM (*8*). However, bacteria maintain a basal level of mercury reductase activity that is able to efficiently reduce even very low concentrations of Hg^{2+} to Hg^0 (*14*), and the expression of mercury reduction activity by bacteria is dependent on factors other than mercury concentrations in water (*15*). In addition to theoretical arguments completely discounting microbial DGM production, investigators observed that DGM was produced in filtered (0.7 μm) lake water at rates comparable to unfiltered water (*5*) suggesting that microorganisms were not a significant source of DGM production in lake water. Many bacteria in oligotrophic environments are well-known to have diameters <0.7 μm and could pass through the filters used in that study. Thus, the role of microorganisms in DGM production in pristine freshwaters cannot be discounted.

We postulate that in addition to being a source of DGM, microbial activity may also reduce levels of DGM in lake water. Bacterial enzymes induced by H_2O_2, *e.g.* hydroperoxidase-catalase (*Kat*G) and other unidentified catalases, oxidize Hg^0 to Hg^{2+} (*16*). These enzymes are not restricted to a particular group of organisms but are widely distributed among the eubacteria (*16*). The oxidation of Hg^0 to Hg^{2+} has not been shown to be a specific reaction of a distinct catalase but rather may be a nonspecific reaction of microbial catalases. H_2O_2 follows a strong diel pattern with increasing levels of H_2O_2 in lake water typically occurring in late afternoon (*17*, *18*), but H_2O_2 is not capable of directly reducing Hg^{2+} to elemental mercury (*19*). H_2O_2 induces bacterial catalase activity because bacteria need to protect themselves from the harmful effect of H_2O_2 on intracellular processes. This induction may occur through a stimulation of genetic transcription, e.g. via *oxyR*, or an increase in catalase activity may be the result of increasing the cosubstrate, H_2O_2, for the catalase reaction. Hence, the induction of microbial oxidase activity by H_2O_2 is a possible explanation for the decrease in DGM levels observed in freshwater lakes during the afternoon. In this study, we investigated the role of microbial activity and its interaction with photochemical processes, in the diel patterns of DGM in lake water.

Experimental Section

Site Description. We measured DGM concentrations every 45−90 min as well as microbial mercury reductase and oxidase activity in three bays at Jack's Lake, located approximately 200 km northeast of Toronto, Canada

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(44°41′20″ N, 72°02′54″ W) on July 21, 22, and 23, 2000 and in Lake Ontario during the week of September 10−14, 2000. Similar weather prevailed over the 3-day period of July 21, 22, and 23 at Jack's Lake with no rain occurring and each day being sunny and clear. Jack's Lake, which has been the site of detailed H_2O_2 studies (*17*, *18*, *20*−*22*), is a mesotrophic lake on the edge of the Canadian Shield with an average of 14 mg of Ca^{2+} L^{-1}, 12 μg P L^{-1} and pH of 7.2 (*21*). Brookes Bay is shallow, colored, with a dissolved organic carbon (DOC) concentration of 7.8 mg C L^{-1} and shows pronounced anoxia below 7 m with a shallow mixing depth of 3.5 m. In contrast, Sharpes Bay is clear, DOC of 4.6 mg C L^{-1}, and shows little hypolimnetic oxygen depletion to the max depth of 43 m. Williams Bay has features that are between the other bays, DOC of 6.0 mg C L^{-1}, but has metalimnetic peaks of chlorophyll and sulfate reducing bacteria (*23*). Together these three bays which are present in a single lake represent the physical features of most temperate lakes.

Analysis of Dissolved Elemental Mercury in Lake Water. Samples of Jack's Lake water were collected from a fiberglass boat 15 cm below the surface by placing a narrow mouth Teflon bottle directly into water by gloved hand. Samples of Lake Ontario water were collected using a Go-Flo sampler from 1 m depth. DGM was analyzed by bubbling approximately 20 L (1 L min^{-1}) of mercury free air produced by a Tekran Zero Air Generator through a 1 L water sample contained in a closed glass graduated cylinder. The bubbled gas was analyzed for elemental mercury using a Tekran 2537A with precleaned Teflon lines and connections. This instrument first amalgamates mercury onto a pure gold cartridge and then thermodesorbs this mercury, which is analyzed by cold vapor atomic fluorescence spectrophotometry every 5 min. This closed analytical system removes 99% of the DGM from a 1 L sample within 20 min with a daily detection limit of 5−25 fM. Daily detection limit was determined as three times the standard deviation of blanks. The average coefficient of variation of 17 samples analyzed in triplicate was 13%. No diatomic volatile Hg species have been reported in freshwaters. Consequently, values are expressed in fM based on the assumption that only monoatomic Hg species were detected with this instrument. After analysis of DGM, 500 mL of lake water was combined with 100 mL of glycerol, and the samples were frozen for microbial analysis.

Analysis of Microbial Mercury Reductase and Oxidase Activity. Microorganisms were centrifuged from 1 L of unfiltered lake water and lysed, and the amount of protein extracted was quantified (*24*). Protein extracted varied between 95 and 170 μg L^{-1} throughout the 3-day sampling period. Protein, i.e. enzyme, extracts were assessed for their mercury reductase activity (*25*), with a U equivalent to 1 μmol of NADPH consumed in response to an aliquot (20 nmol) of inorganic mercury. Microbial oxidation of elemental mercury was measured using 1 mL additions of water saturated with Hg^0 to 200 μL enzyme extracts of lake water (*16*). Enzyme extracts were incubated at 22 °C for 1 h and a U designated as 10 fmol of inorganic mercury formed. Boiled controls were prepared by heating enzyme samples (100 °C) for 10 min, and background mercury oxidation is subtracted from the reported value. The oxidation of Hg^0 was found to be proportional to the amount of enzyme.

Hydrogen Peroxide Experiments. DGM concentrations for Lake Ontario were made at seven different stations encountered over the 5-day cruise. Weather patterns varied considerably over this time. Lake Ontario surface (sampler was set to 1 m) water was collected with a GoFlo Sampler from Stations 9 (43°35′12″ N, 79°23′42″ W), 81(44°01′00″ N, 76°40′18″ W), and 752 (43°29′55″ N, 79°28′58″ W) in the morning for the H_2O_2 experiments, and station 83 (44°00′00″ N, 76°50′36" W) was sampled around 11 a.m. for the sunlight incubation experiments. The sunlight incubations were performed on the rear deck on the Limno's cruise vessel between 13:00 until 18:00 on a clear and sunny day with dark samples kept inside a cupboard on board. Isolated samples were spiked with 100 nM H_2O_2 that is a typical mid-day concentration of H_2O_2 in Lake Ontario (*26*). DGM, mercury reductase, and oxidase activities were determined every 30 min for 5 h. Data were normalized to the DGM concentrations observed immediately (<5 min) after lake water was spiked with 100 nM H_2O_2. Lake water was filtered in an attempt to determine the relative contribution of abiotic and biotic processes on DGM production. Lake water was filtered through a 0.22 μM syringe top Sterivex filter (*27*) and protein concentration (*28*) used to detect biological activity.

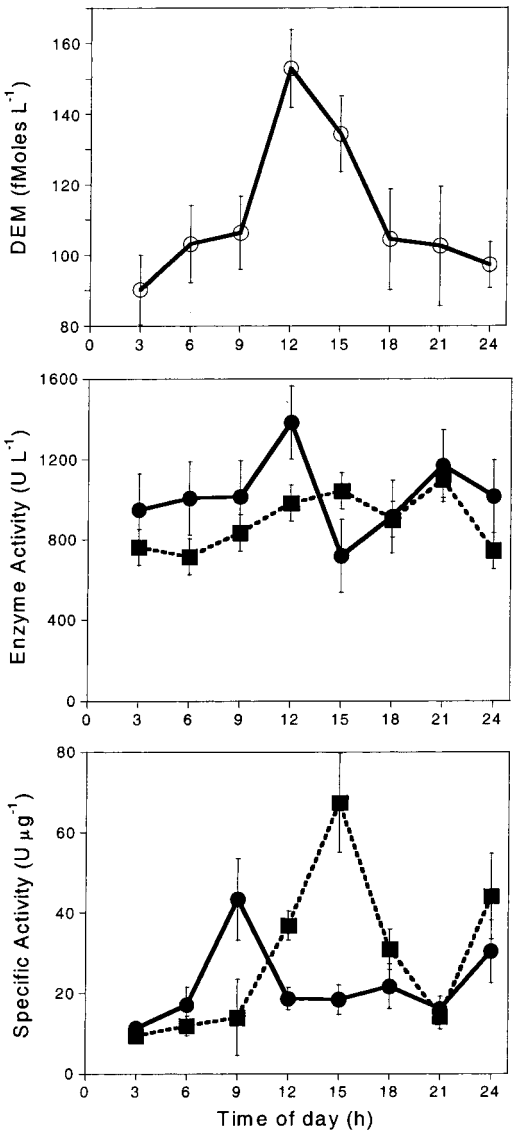

FIGURE 1. Diel pattern of DGM (○), mercury oxidase (■), and reductase (●) activity extracted from three bays in Jack's Lake on July 21, 2000. Each data point is the average of three bays that were measured in duplicate ($n = 6$), and error bars represent the standard error of the estimate.

Results and Discussion

DGM concentrations in all three bays in Jack's Lake peaked at ca. 159 fM ($n = 6$, standard deviation of 26%) at 12:00 and decreased to ca. 90 fM, by 18:00 for the remainder of the day (Figure 1). These DGM concentrations are similar to that observed in Ranger lake (*5*) where experiments on mercury photoreduction were made. Microbial reductase and oxidase

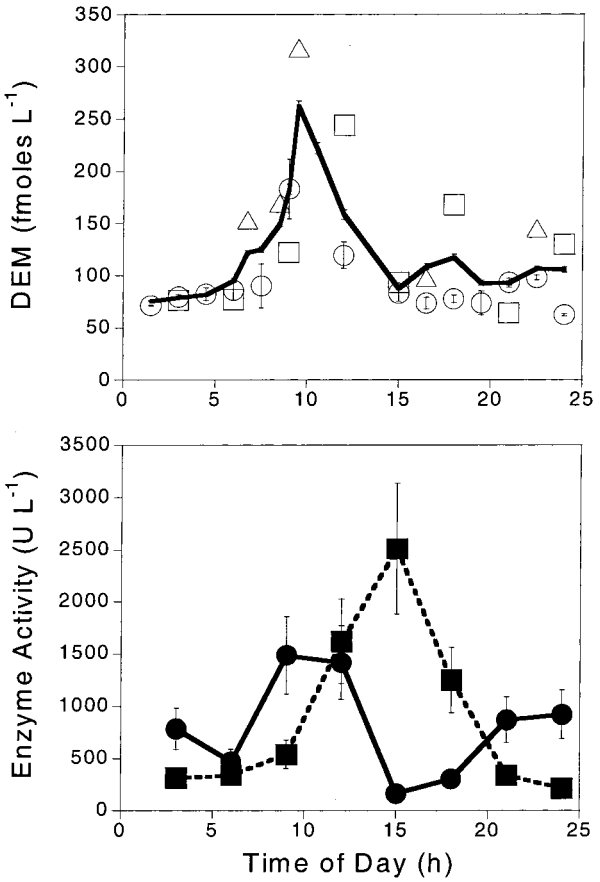

FIGURE 2. Diel pattern of DGM, mercury oxidase (■), and reductase (●) activity over a three day period in Brookes Bay, Jack's Lake. Each data point for DGM for July 21 (△) and July 22 (□) is a single sample with duplicate samples analyzed on July 23 (○). The moving average (—) is the average of observations taken over the 3-day period immediately before, at, and after the indicated time of sampling with error bars indicating the standard error of this estimate. Each data point for microbial mercury oxidase and reductase activity is the average of duplicate samples taken on July 22 and 23.

activity patterns were similar in all three bays with a strong increase in mercury reductase activity in the morning followed by mercury oxidase activity peaking in late afternoon. Similarly, DGM concentrations in Lake Ontario were the highest before noon peaking at 736 fM (standard deviation of 209 fM, $n = 3$) and the lowest, 284 fM (standard deviation of 100 fM, $n = 4$), in the afternoon. This pattern is also similar to that previously observed in Ranger lake (*5*) and the Florida Everglades (*4*) suggesting that we are observing a general pattern of DGM in the uppermost surface water of freshwaters lakes. We did not sample with depth across the upper mixed zone, but future studies should do so as mixing may account for part of the DGM decline after noon. Levels of DGM in Lake Ontario were similar to that previously observed, 154−667 fM (*29*), and are somewhat higher than that previously observed, 145 ± 85 fM $n = 5$, in Lake Michigan (*12*), but it is not clear at what time of day the Lake Michigan samples were collected.

Diel patterns of DGM in Brookes Bay (Figure 2) were highly reproducible over a 3-day period peaking between 10:00 and 12:00 followed by a steady decrease to the baseline concentration of DGM of ca. 100 fM by 16:00. Mercury reductase activity paralleled DGM levels in lake water with a sharp increase in reductase activity occurring in the early morning, peaking by 12:00, and decreasing by 15:00. Mercury oxidase activity, i.e. DGM consumption, did not significantly increase until later in the day, peaking at 15:00. Mercury reductase activity increased again after 15:00, but at that point mercury oxidase activity was elevated, and thus, the net result on DEM concentrations was minimal. The regulation of mercury reductase activity at environmental mercury concentrations and in in situ communities is an area that requires further research. The strong reproducibility of the diel patterns in Brookes bay supports our assertion that these diel patterns are a general phenomenon of freshwater lakes.

The diel pattern of mercury oxidase activity observed in all three bays as well as Lake Ontario was similar to previously published diel patterns of hydrogen peroxide (*21*, *17*). However, no H_2O_2 additions were made at Jack's Lake because no effect on DGM levels measured 4 h after H_2O_2 addition was observed in samples from Ranger Lake (*5*). At that time, DGM analysis was more time-consuming, and thus, it was not possible to develop more detailed time course data. The strong similarity between H_2O_2 and DGM patterns convinced us that H_2O_2 may be related to DGM concentrations in water but that it may occur over a time scale, that previous investigators were unable to determine. Previous work on Jack's lake demonstrated that bacteria are principally responsible for H_2O_2 decay and that bacterial abundance is highly correlated with decay rates of H_2O_2 (*17*, *18*, *20*−*22*), and thus, we postulated that the H_2O_2 patterns may also influence other bacterial activities, such as the regulation of DGM. Additions of H_2O_2 increased mercury oxidase activity by 250% within 30 min, and 60 min after H_2O_2 addition, DGM had decreased to 28% of the initial value (Figure 3). At time 0, unfiltered samples contained 390 fM (SD of 95 fM, $n = 3$) DGM and for filtered samples 467 fM (SD of 130 fM, $n = 3$) DGM which is well within the range observed at other sampling stations on Lake Ontario. At time 0, there was 1060 U L^{-1} mercury reductase and 720 U L^{-1} mercury oxidase activity in unfiltered water but only 58 U L^{-1} mercury reductase and 60 U L^{-1} mercury oxidase activity in filtered water. Hydrogen peroxide may increase mercury oxidase activity by either inducing peroxidase enzymes such as *katG* or alternatively, by increasing the amount of intracellular cosubstrate for the oxidation of Hg^0 (*16*). In a manner similar to that observed at Jack's Lake, mercury reductase activity and DGM concentrations in water paralleled one another with only a short time lag of 30 min occurring between the increase in mercury reductase activity and an increase in DGM concentrations. This increase in mercury reductase may be related to the stimulation of mercury reductase activity by the increase in divalent mercury (Hg^{2+}), ca. 246 fM, in the environment. DGM stabilized by 240 min at levels comparable to that initially seen.

Previous investigator's DGM data, measured 0 and 240 min after H_2O_2 addition, does not reflect the remarkable transformations which may take place over this 4 h period (*5*). Hydrogen peroxide induced DGM fluctuations were not observed in filtered lake water. Therefore changes in DGM concentrations in nonfiltered lake water were a result of biological activity and confirm earlier work that H_2O_2 is not capable of reducing Hg^{2+} to elemental mercury (*19*). The H_2O_2 spiking experiments implicate microbial oxidation activity in the regulation of DGM concentrations in lake water. The sine-wave pattern observed with the H_2O_2 spiking experiments was not observed during the diel patterns and is likely due to the continuous production of H_2O_2 throughout the peak periods of the day. These experiments illustrate that a cascade of microbial driven mercury transformations is triggered by H_2O_2 production in lake waters. From our results it is not clear the mechanism by which H_2O_2 induces mercury oxidase activity. It could be the induction of the mercury oxidase gene as observed in pure culture studies (*16*). Alternatively, the increase in electron acceptor concentrations for the catalase reaction, in this case H_2O_2, may

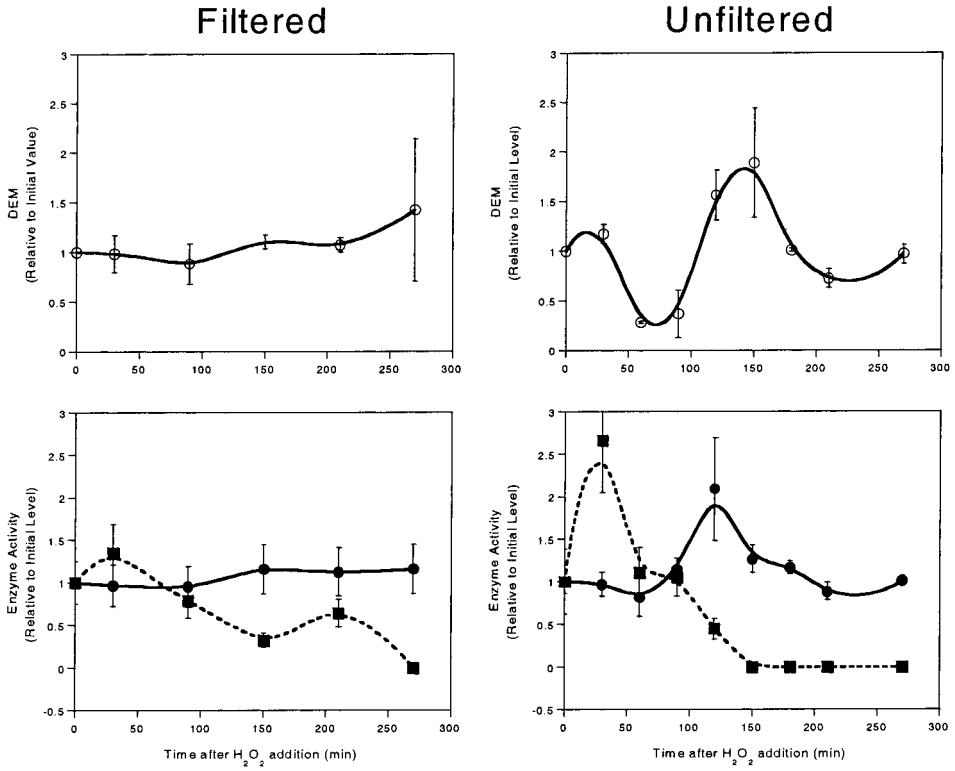

FIGURE 3. Induction of microbial mercury oxidase (■) and reductase (●) activity and resulting DGM (○) concentrations by the addition of H_2O_2 to Lake Ontario water. Each data point for DGM is the average of independent incubations from three different sampling locations on Lake Ontario. Each data point for enzymes from unfiltered water is from the three different sampling locations but for filtered water, it is the average of two different sampling locations only.

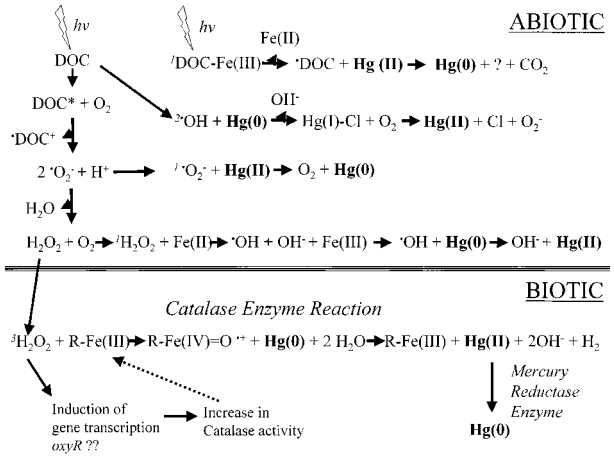

FIGURE 4. Conceptual diagram outlining the importance of sunlight for the two known biological and four known abiotic transformations of divalent and elemental mercury in freshwaters: [1]-reactions described by Zhang and Lindberg (*9*), [2]-reactions described by Lalonde et al. (*30*), and [3]-reactions described in this study. The relative importance of each reaction pathway has yet to be determined.

result in an increase in the oxidation of mercury by preexisting catalase's present in the water column.

Our work illustrates that H_2O_2 produced by solar radiation stimulates mercury oxidase activity in lake water, which results in a decrease in DGM levels during the afternoon. Although we did not determine in situ activity of mercury oxidase, we do illustrate that the potential mercury oxidase activity increases in response to H_2O_2 produced by solar radiation. Further, our results illustrate the interplay of microbial reduction and oxidation activities with photochemical processes in controlling levels of DGM in surface water of lakes. This interplay is illustrated in Figure 4. Irradiation of lakes begins a cascade of photochemical reactions that in turn trigger four known abiotic and two known biotic transformations involving elemental mercury. Important cofactors in these reactions such as benzoquinones and Fe(III) are just now beginning to be uncovered by investigators (*9*, *30*). The relative contribution of photoreduction and photooxidation must now be placed in context with reduction and oxidation rates due to microbial activity in order to develop an accurate model for DGM levels in surface waters. Future investigations will focus on a comparison of actual in situ rates of photooxidation/reduction to in situ rates of microbial mercury oxidation and reduction.

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