

Biological and photochemical production of dissolved gaseous mercury in a boreal lake

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Abstract

We used in situ experiments and measured depth profiles of dissolved gaseous mercury (DGM) to investigate the relative contribution of photochemical versus biological processes on the production of DGM in an oligomesotrophic lake of the Canadian Shield. At the surface, DGM production was mainly photomediated, with reduction rates being twice as high in the wetland than in the lake. In the water column, the distribution of DGM concentrations was not strictly related to light but followed a multimodal distribution, with peaks encountered below the epilimnion at depths receiving <5% of the incident light. Those peaks were recorded in the middle and at the bottom of the metalimnion during the ice-free season, as well as under ice cover and at the bottom of an anoxic hypolimnion. Rather than being a consequence of the bacterial mercuric reductase activity, metalimnetic DGM peaks were associated with the intensity and duration of phytoplankton blooms. In situ incubation experiments also showed that DGM production ceased when samples were kept in the dark, filtered, or when an inhibitor of photosynthesis was added. Our results illustrate the important role of phytoplankton on Hg redox dynamics in the water column of lakes.

Hg(0), which is emitted by natural sources or from power plant facilities and incinerators, travels over long distances in the atmosphere and, once it is oxidized to Hg(II), is deposited in remote areas far from its emission source (Iverfeldt and Lindqvist 1986; Swain et al. 1992; Schroeder and Munthe 1998). Once it is methylated in aquatic ecosystems, Hg is bioaccumulated in food webs, with levels a million times greater in predatory fish than those found in the water

column, which causes a significant health risk to populations whose diet relies on fish.

Alternatively, Hg(II) deposited onto water bodies can be reduced back to volatile Hg⁰ and transferred to the atmosphere (Ebinghaus et al. 1999). This Hg reduction may alter the pool of Hg within aquatic systems by competing with the methylation process for Hg(II) substrate (Fitzgerald et al. 1991). However, the exact relationship between Hg levels in the atmosphere and in fish remains unclear. In this context, an initiative called METAALICUS (Mercury Experiment to Assess Mercury Loadings in Canada and the United States) aims at clarifying this relationship by adding stable isotopes of Hg to a whole ecosystem and monitoring its fate over time. Here, we present results on the relative contribution of the photochemical versus biological formation of dissolved gaseous mercury (DGM; mainly Hg⁰) in the spiked ecosystem.

The production of DGM [which is mainly formed by the reduction of Hg(II) to Hg(0)] can be photomediated (Amyot et al. 1994; Krabbenhoft et al. 1998; O'Driscoll et al. 2003). UV-A and visible radiation are involved in waters with high concentrations of dissolved organic carbon (DOC) and UV-B radiation in clear-water lakes, such as pristine lakes of the High Arctic (Amyot et al. 1997*a,b*). Although, in most instances, peaks of DGM have been reported at the air-water interface, high DGM concentrations have also been occa-

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This is contribution no. 8 of the METAALICUS.

Table 1. Characteristics of Lakes 658 and 240.

Parameters	Lake 658	Lake 240
Surface area (ha)	8.34	44.1
Wetland area (ha)	1.9	—
Maximum depth (m)	13	13
Water residence time (yr)	4–5	~1.5
pH	6.65	6.8
Dissolved organic carbon (mg L ⁻¹)	9	7
Top predatory fish	Northern pike (<i>Esox lucius</i>)	Northern pike (<i>Esox lucius</i>)

sionally observed at deeper depths of lakes (Vandal et al. 1991; Mason et al. 1995; Amyot et al. 1997a), where photochemical reactions are unlikely to play a major role. Biological Hg reduction has been invoked to explain DGM patterns in the Pacific Ocean (Mason and Fitzgerald 1993; Baeyens and Leermakers 1998) and in freshwater and estuarine environments (Vandal et al. 1991; Mason et al. 1995; Siciliano et al. 2002). Laboratory and in situ experiments in contaminated waters have shown that microbes may use an enzymatic pathway that expresses the mercuric reductase gene (*merA*; Barkay et al. 1989, 1991) to convert inorganic divalent Hg to elemental Hg. More recently, work on Canadian Shield lakes has correlated reductase activity in freshwaters to DGM concentrations (Siciliano et al. 2002). Phototrophic organisms, such as algae, can also reduce Hg, a phenomenon that is thought to be related to photosynthesis (Ben-Bassat and Mayer 1978).

The purpose of the present article is to further investigate the role of microorganisms—namely phytoplankton—on Hg cycling, in a lake amended with isotopic Hg at natural levels. We monitored the distribution of volatile forms of Hg within the water column of Lake 658 (Experimental Lakes Area, Ontario, Canada). We also investigated the relative contribution of photoreduction versus biological reduction in DGM formation through a series of incubation experiments at the lake surface, as well as deeper in the water column. Results indicated that DGM production was mainly photo-mediated, not biologically mediated, at the surface. In contrast, at depth, DGM production was linked to the presence of algae and was enhanced during metalimnetic phytoplankton blooms.

Experimental section

Study site—Sampling and incubation experiments were conducted in or near Lake 658 (49°43.9'5N, 93°44.2'W) in the Experimental Lakes Area. Lake 658 is a double-basin

headwater lake. The depths of the respective east and west basins are 9 and 13 m. It is a mesotrophic, humic lake (Table 1). As a control for this whole ecosystem experiment, Lake 240 was chosen as a reference (Table 1).

Isotopic addition—General information regarding the spike is summarized in table 2. Three different stable isotopes of Hg (¹⁹⁸Hg, ²⁰¹Hg, and ²⁰²Hg) were spiked on the three different components of the ecosystem—the lake, its wetland, and its surrounding upland.

Twenty-four hours before each lake spike, 2 × 20 liter plastic jars were filled with water from Lake 658 and stored in the dark. A few hours before the spike, isotopes were added to each container. The lake was spiked biweekly at dusk at a depth of 0.7 m, to prevent any evasion during the spike. Both basins of Lake 658 were spiked at the same time, using boats powered by electrical motors. The application rate was fixed at 2.4 μg m⁻² per spike, which translates into a yearly application rate of 21.6 μg m⁻² yr⁻¹ (see also Table 2). Total Hg concentrations averaged 16.6 ± 2.8 pmol L⁻¹ during 2001 and 2002. In this article, we only present data on total DGM, not isotopic DGM.

Sampling and analysis of DGM—Glassware and Teflon bottles were thoroughly washed with acid, and nonpowdered gloves were worn at all time. Surface samples were hand filled (with no headspace) from a nonmetallic boat. For deeper samples, water was pumped from the depth of interest using a neoprene-coated Teflon line and a peristaltic pump (Masterflex). On the boat, the line was thoroughly washed with HCl 20% (v/v) before collection by continuously circulating the acidic solution through the line for 5 min. The line was then carefully rinsed with lake water at each depth before samples were taken from that depth. In 2001, to monitor DGM concentrations close to the sediment-water interface, we used the close-interval sampling system (Babiarz et al. 2003). This device allowed for the sampling of water

Table 2. Characteristics of the lake Hg spikes for years 2001 and 2002.

Characteristic	Measurement
Stock solution	²⁰² Hg (purity 90.8%) in HNO ₃ 5%
Application rate (μg ²⁰² Hg m ⁻² yr ⁻¹)	21.6
Lake surface area (ha)	8.34
Amount per spike (g Hg)	0.2
Application dates	2001: 19 Jun, 3 and 17 Jul, 1, 15 and 28 Aug, 11 and 25 Sep, and 9 Oct; 2002: 4 and 18 Jun, 2, 16 and 30 Jul, 13 and 27 Aug, 10 and 24 Sep

at a fixed, fine scale (5, 10, 20, 40, and 80 cm above the sediment-water interface). Sampling ports on the fixed sampler located at the bottom of the lake allowed temporal sampling of exact depths, regardless of conditions that would affect a sampling boat.

Note that some DGM may have been lost through oxidation between the time of collection and laboratory analysis (a delay of ~3 h). Because Lalonde et al. (2001) have shown that such an oxidation was favored by solar radiation, we minimized these losses by preventing any unwanted exposure of the samples to sunlight. We also included dark controls in all our experiments, to determine whether dark oxidation occurred; these controls consistently showed that dark oxidation was not a significant process during short-term (<10 h) experiments.

In the laboratory, each sample (500 ml) was cautiously poured in a 1-liter glass-ambered bubbler and sparged for 20 min with ultra-high-purity argon. Elemental Hg was trapped on gold wire/sand traps (Brooks Rand). Gold traps were then desorbed by pyrolysis and quantified by gas-phase atomic fluorescence spectrometry (Tekran model 2500) using the double-amalgamation technique. The detection limit of the method was 5 fmol L⁻¹ and was calculated as three times the SD of 10 replicates with low DGM concentrations. Triplicates were regularly taken, and they typically varied between 1% and 6%. Bubbler blanks (2–4% of DGM in samples) were subtracted from the total DGM concentrations. We did not distinguish between elemental Hg and volatile organic Hg (i.e., dimethyl Hg), because other studies have consistently shown that volatile organic species are very rare in remote freshwater lakes (Vandal et al. 1991).

Levels of DGM reported in the present article are somewhat higher than those usually reported for pristine temperate systems (Amyot et al. 1997; O'Driscoll et al. 2003). This is mainly due to the addition of Hg(II) to the water column, which nearly doubled the value of total Hg in the system (from 10 to 18 pmol L⁻¹) and likely increased the pool of photoreducible Hg. To confirm that these relatively high values were not due to analytical errors, we performed the following quality controls. First, we conducted an intercalibration of elemental Hg measurements with four other independent laboratories. Known amounts of aqueous Hg⁰ were trapped on gold traps and shipped to the different laboratories. Our values were, on average, within 12% of the expected values. We also compared our DGM levels with those measured at the same site during overlapping sampling seasons by an independent team from Oak Ridge National Laboratory (ONRL; Southworth and Lindberg pers. comm.). From June to August 2001, we measured an average DGM concentration of 0.81 ± 0.33 pmol L⁻¹ (*n* = 45 weekly measurements). The ONRL team made two samplings for DGM—one in June and the other in August 2001. Their average DGM levels for June and August were 0.75 ± 0.56 pmol L⁻¹ (*n* = 29) and 1.30 ± 0.38 pmol L⁻¹ (*n* = 18), respectively. Our average DGM values are therefore well within the range of values measured independently by ONRL.

Incubation experiments—For in situ reduction experiments at depth, water collected at night was incubated at the

depth of interest in Teflon bottles using plexiglass supports. These supports were attached to a polypropylene line tied to a styrofoam buoy coated with polyethylene terephthalate (PET). All manipulations (sampling, filtration, and the addition of the inhibitor) were performed at night and on the boat at the center buoy.

In experiments that used surface water, samples collected at night were incubated on a deck, with their mouth pointing north, in a container continuously filled with surface water, to keep the temperature constant. After incubation, bottles were kept in the dark until analysis. Incubation under sunlight was carried out from 1000 to 1500 h for the wetland water (07 August 2001) and from sunrise to noon for the lake water (02 August and 28 September). DGM was measured over the time course of the experiments at a rate of one bottle per hour.

Some samples were filtered before incubation. Filtration was done online, in the field, using either 0.1 or 1 μm porosity inline Whatman filters (phytoplankton organisms were mainly colonials and with a size >1 μm).

To stop photosynthesis, some bottles were spiked with dichlorophenyldimethyl urea (DCMU). A 10⁻³ mol L⁻¹ stock solution of DCMU was prepared by dissolving 2.33 mg of DCMU (Sigma Aldrich) in 100 ml ethanol. The final DCMU concentration in incubation bottles was 25 μmol L⁻¹. To test the influence of DCMU on Hg speciation, we incubated milli-Q water with DCMU and DGM for 3 d in the dark. No change was observed in DGM levels. Although the chemistry of DCMU-Hg interactions has not been precisely described in the literature, our results suggest that DCMU does not affect oxidation and reduction directly.

DGM production rates—We assumed that the rate expression (*v*) for DGM production is

$$v = -d[\text{Hg}_p]/dt = k \cdot [\text{Hg}_p]^\alpha \cdot [\text{photored}]^\beta \quad (1)$$

where [Hg_p] and [photored] represent the concentration of photoreducible Hg and photoreductants and α and β represent the order with respect to mercury and photoreductant, respectively. Because we did not measure [photored], eq. 1 is simplified as

$$v = -d[\text{Hg}_p]/dt = k' \cdot [\text{Hg}_p]^\alpha \quad \text{with } k' = k \cdot [\text{photored}]^\beta \quad (2)$$

where *k'* is the apparent constant. Under the assumption that α is equal to unity and the concentration of photoreductant remains constant in the course of reaction (e.g., if the photoreductant concentration is in excess), equation (2) holds. From equation (2), we obtain

$$\ln[\text{Hg}_p]_t = \ln[\text{Hg}_p]_0 - k't \quad (3)$$

We assume that the initial amount of photoreducible Hg, [Hg_p]₀, corresponds to the DGM concentrations reached at the plateau, during short-term incubation experiments under sunlight. [Hg_p]_t represents the amount of Hg still photoreducible at time *t*, expressed as the difference between [DGM] obtained at the plateau after 4–10 h of incubations and the [DGM] at time *t*.

Sampling for microorganisms and water chemistry—Integrated epilimnetic and metalimnetic samples for bacterial

and phytoplankton analysis were collected using an integrating sampler (Shearer 1978). Samples were then transferred in 100-ml glass bottles and preserved with 2% formalin (bacteria biomass) and 1% lugol (phytoplankton biomass) and stored in the dark at 4°C.

For water chemistry, water was collected at depths of 2, 5, 7, 9, 11, and 13 m using a Masterflex line connected to a propeller pump (SUNCAN). Samples for DOC and chlorophyll were then filtered through Whatman GF/C filters. Filtrate samples were analyzed for DOC with an automated TOC analyzer (model 700; O.I.). Chlorophyll filters were dried, frozen, and stored in the dark until analysis. They were then extracted with a methanol-acetone solution, and chlorophyll was analyzed with a Shimadzu fluorometer. Oxygen measurements were done using the Winkler titration.

Irradiance measurements—Irradiance profiles were done using an Optronics spectroradiometer recording the whole light spectrum at discrete wavelengths from 300 to 800 nm. Irradiance was measured at the surface and at depths of 0.2, 0.5, and 1 m. Continuous photosynthetically active radiation (PAR) measurements were obtained using a Li-Cor Li-1400 logger with a Li-Cor Li-190SA sensor.

Phytoplankton analysis—The method for phytoplankton enumeration is fully described in Findlay et al. (2001). In brief, the method uses the Ütermohl technique as modified by Nauwerck (1963). Cell counts were converted to wet weight biomass by approximating cell volume. The mixotrophic state of some of the cells was confirmed by microscopic observation. Indeed, mixotrophs lack characteristic chloroplasts. Samples for bacterial counts were stained with 4,6-diamidino-2-phenylindole (Pomroy 1984) and analyzed using epifluorescence microscopy.

Collection of microbial biomass, RNA extraction and quantitation, and cDNA synthesis—Microbial biomass was collected by filtration of water samples onto 0.22 µm Sterivex-GS filters (Millipore). Filters were immediately placed on dry ice and kept frozen until analysis. Total RNA was extracted from filtered aquatic biomass according to the method of Jeffrey et al. (1996). Contaminating DNA was removed by treatment with RQ1 DNase (Promega), according to the manufacturer's instructions, and the concentration of RNA in DNase-treated extracts was quantified as described in Jeffrey et al. (1996) against a calibration curve prepared with *Escherichia coli* W rRNA (Sigma-Aldrich). Reverse transcription (Superscript II; Invitrogen Life Technologies) was used to convert RNA to cDNA according to the manufacturer's instructions.

Small subunit rRNA clone libraries—Methods described by Priemé et al. (2002) were used with some modifications (see below) to construct and analyze 16s rRNA clone libraries representing the active bacteria in water samples collected at depth of 5.5 and 12 m in September 2001. Modifications included (1) the use of 16 rRNA-specific primers (Weisburg et al. 1991) for polymerase chain reaction (PCR) amplification from cDNA, (2) the use of the TOPO TA Cloning Kit (Invitrogen) for cloning and sequencing of 16s rRNA

amplification products, and (3) the use of the restriction enzymes *MspI* and *HeaIII* (Promega) for restriction fragment-length polymorphism (RFLP) analysis. Sequencing was carried out as described by the manufacturer (TOPO) on an ABI 3100 genetic analyzer (Applied Biosystems) at the Biotechnology Center for Agriculture and the Environment, Rutgers University. Identification of the nearest relative of the bacteria of origin was obtained by BLASTN analysis of 16s rRNA sequence data available at <http://www.ncbi.nih.gov/>.

Detection of merA mRNA transcripts—Attempts to detect *merA* transcripts in microbial biomass samples collected at depths of 5.5 and 12 m in Sep 2001 were carried out as described elsewhere (Nazaret et al. 1994), except that PCR amplification from cDNA using *merA*-specific primers replaced Northern hybridization. The PCR primers, A7s-n91.F (5'-CGATCCGCAAGTGGCIACBGT-3') and A5-n54.R (5'-ACCATCGTCAGRTARGGRAAVA-'), were designed to span the known diversity of *merA* sequences from gram-negative bacteria, producing an amplicon of 288 bp (Barkay unpubl. data). PCR was done with 25-µl, 1× reaction buffer, 100 ng cDNA, 1.5 mmol L⁻¹ MgCl₂, 0.2 mmol L⁻¹ dNTPs, 0.6 µmol L⁻¹ primers, and 0.5 U Taq polymerase (Fisher). Amplification conditions were 45 cycles of 15 s at 94°C and 60 s at 60°C. Each cDNA sample was first amplified with 16s rRNA-specific primers, to verify the presence of cDNA of microbial origin, followed by the detection of glutamine synthase transcripts (*glnA*), an abundant transcript in microbial community RNA extracts (Hurt 2001), to verify the presence of intact mRNA transcripts. This approach and a detailed description of the methods will be published elsewhere (Yagi and Barkay unpubl. data).

Results

DGM production at the surface—When surface-water samples were incubated under the sun, DGM production only occurred in transparent bottles, not in black ones (Fig. 1A–E). DGM concentrations were strongly related to the cumulative PAR and reached a plateau after 3–5 h of incubation (Fig. 1A–E). DGM formation rates (k' values) were higher in the wetland than in the pelagic area [$k' = 0.76$ – 1.4 h⁻¹ in the wetland (Fig. 1D,E) and 0.21 – 0.47 h⁻¹ for the pelagic area (Fig. 1A–C)], although the hourly absolute rate of photoproduction was higher in the pelagic area ($[DGM]_{\text{prod}} = 0.089$ – 0.101 pmol L⁻¹ h⁻¹ in the wetland and 0.110 – 0.281 pmol L h⁻¹ in the pelagic area). The concentration of DOC in mid-July 2001 was twofold higher in the wetland (20 mg L⁻¹) than in the lake (9 mg L⁻¹).

Total Hg levels were 10 pmol L⁻¹ before the initial Hg(II) spike in Lake 658 and reached a maximum value of 18 pmol L⁻¹ at midsummer. This is to be compared with DGM values in the lake of 0.4 – 1.2 pmol L⁻¹. In closed bottles exposed to solar radiation, DGM levels reached maximum values of ~ 2 pmol L⁻¹. The levels of Hg(II) present in lake water were therefore about an order of magnitude higher than DGM levels, which is consistent with the literature (Morel et al. 1998).

Filtration (porosity = 0.1 µm) of pelagic surface water samples before incubation caused an expected decrease in

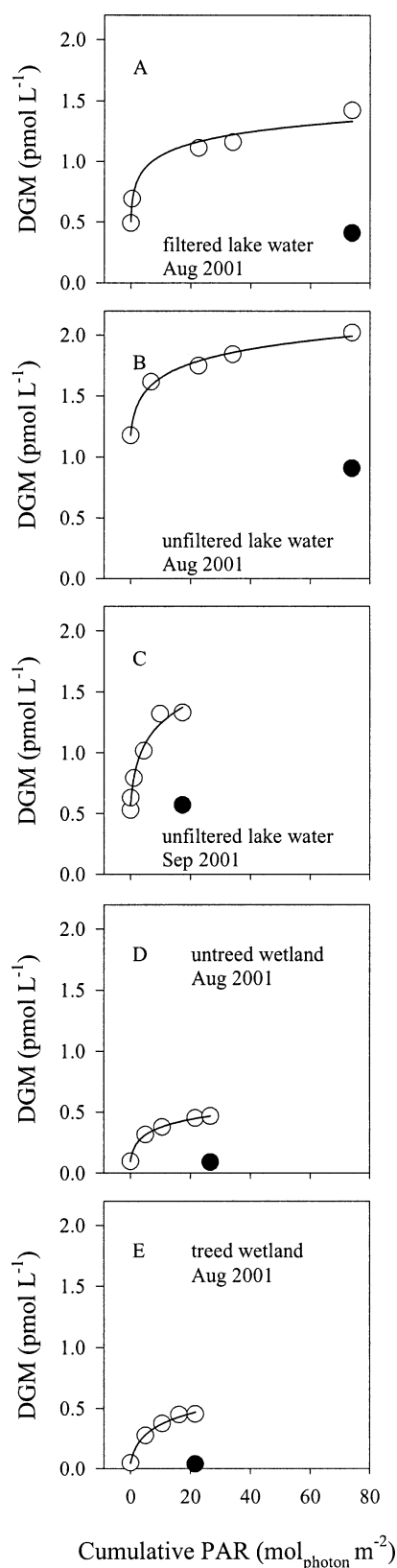


Fig. 1. Change in DGM concentrations during incubation experiments as a function of cumulative PAR (400–700 nm). White symbols represent samples kept in clear bottles, and black symbols represent samples kept in the dark. (A,B) Unfiltered vs. filtered surface

initial DGM levels (Fig. 1A,B). Indeed, to keep the integrity of the living cells and to prevent their inner contents from bursting outside, we vented the in-line filter during the filtration step. However, DGM production rates were similar between filtered ($k' = 0.21 \text{ h}^{-1}$ and $[\text{DGM}]_{\text{prod}} = 0.112 \text{ pmol L}^{-1} \text{ h}^{-1}$) and unfiltered samples ($k' = 0.25 \text{ h}^{-1}$ and $[\text{DGM}]_{\text{prod}} = 0.110 \text{ pmol L}^{-1} \text{ h}^{-1}$) (Fig. 1A,B), which indicates that cells were not carrying out Hg(II) reduction in this surface water.

Seasonal changes in DGM depth profiles—We monitored DGM depth profiles during summer, fall, and late winter (under ice) (Fig. 2A–F). The highest levels were always found in the top 2 m of the water column. A second peak was regularly seen at the bottom of the metalimnion (in August and September 2001 and July 2002). This peak was located near 5.5 m in 2001 and at 3.5 m in 2002, reaching similar values as the surface peaks on two occasions (13 August 2001 and 1 July 2002). Peaks at depth were also observed in March (between 0 and 3 and 9 m; Fig. 2C), when the water column was covered with ice and a thick layer of snow; winter DGM levels were typically 10 times lower than during the ice-free season. Finally, smaller peaks (up to 0.3 pmol L^{-1}) were encountered at the sediment-water interface when the bottom of the hypolimnion was anoxic, especially in 2001 (Fig. 2A,B) ($[\text{O}_2]_{\text{aug2001}} < 0.5 \text{ mg L}^{-1}$, $[\text{O}_2]_{\text{sep2001}} < 0.01 \text{ mg L}^{-1}$). The average oxygen concentration from July to September at depths lower than 9 m was 0.47 mg L^{-1} in 2001 and 7.3 mg L^{-1} in 2002. DGM concentrations behaved in an opposite manner to oxygen concentrations, with hypolimnetic levels 4.7 times higher in 2001 (0.18 pmol L^{-1}) than in 2002 (0.04 pmol L^{-1}) ($p < 0.001$, t -test).

Metalimnetic peaks of DGM during the ice-free season—We monitored the daily and seasonal changes in DGM levels in the metalimnion. On 13 August 2001, we observed a large metalimnetic day peak of DGM that decreased by a factor of six overnight (Fig. 3A). This peak was located at the bottom of the metalimnion, at a depth of 5.5 m, which coincides with maxima in chlorophyll and phytoplankton biomass. It decreased by a factor of four from August to September (Fig. 2A,B).

During early July 2002, we repeated these night-versus-day DGM profiles. Although the DGM peaked again in the metalimnion, we did not observe a decrease at night (Fig 3B). DGM levels were one-half those reported in the metalimnion for the preceding year (Fig. 2A,D). From early to mid-July, metalimnetic DGM concentrations decreased below our detection limit (5 fmol L^{-1}) (Fig. 2E), and they increased again in August, reaching $0.55 \pm 0.04 \text{ pmol L}^{-1}$ (data not shown).

In situ incubation experiments were conducted to assess the role of organisms thriving in the metalimnion, at a depth

←

lake water incubations (Aug 01). (C) Unfiltered surface lake water (Sep 01). (D,E) Unfiltered surface wetland water (Aug 01). Equation of fitted curves: $[\text{DGM}] = [\text{DGM}]_0 + a \ln(\text{PAR} - \text{PAR}_0)$.

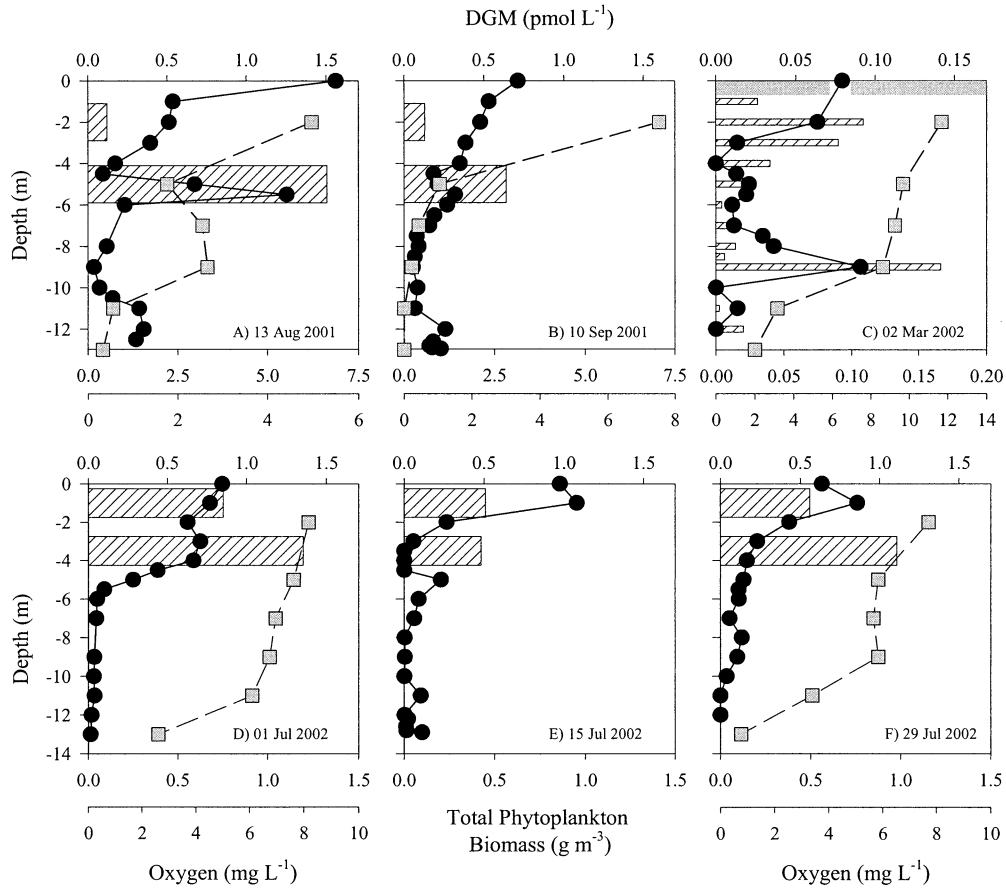


Fig. 2. Seasonal depth profiles in Lake 658. Black circles are DGM concentrations, and the hatched bars represent the epilimnetic and metalimnetic TPB. Gray squares represent oxygen concentrations. Note that TPB data are from integrated samples of the epilimnion and metalimnion in all cases, except in Mar 2002.

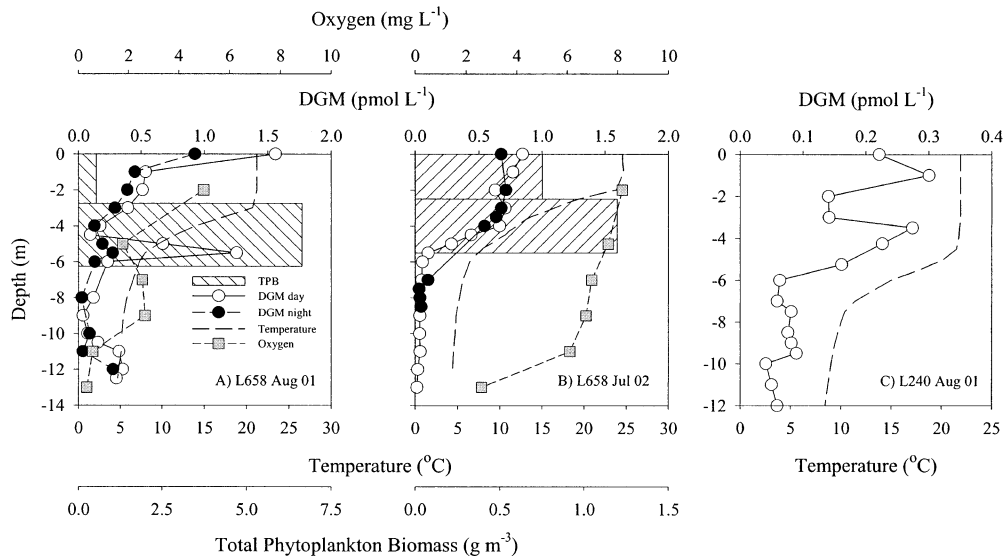


Fig. 3. Diel depth profiles within the water column of Lake 658 in (A) Aug 2001 and (B) Jul 2002 and (C) depth profile in reference Lake 240 (Aug 01).

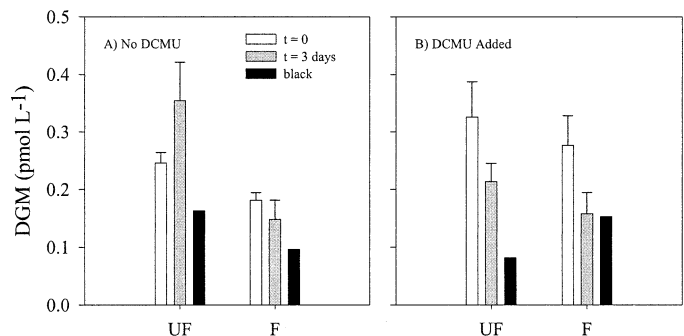


Fig. 4. In situ incubation experiments in Lake 658 at a depth of 3.5 m on 03 Aug 02. Open bars represent initial DGM concentrations, gray bars represent DGM concentrations after 3 d of in situ incubations, and black bars represent the black controls. (A) Treatments with no DCMU added. (B) Treatment with DCMU added to reach a final concentration of $25 \mu\text{mol L}^{-1}$. F = filtered, UF = unfiltered.

of 3.5 m. DGM concentrations increased by 33% in the unfiltered treatments and decreased by 19% in the filtered treatment after 3 d of in situ incubation (Fig. 4A). When DCMU was added to the samples or when samples were kept in continuous darkness, DGM concentrations decreased in both filtered and unfiltered treatments (Fig. 4B).

To establish whether these metalimnetic peaks of DGM were also seen in other systems, we measured a depth profile in Lake 240, at the Experimental Lakes Area (Fig. 3C). Similar to Lake 658, a DGM peak was located at the bottom of

the epilimnion and corresponded to the very bottom of the euphotic zone.

Phytoplanktonic and bacterial dynamics—In August 2001, an important peak of chlorophyll reaching $27 \mu\text{g L}^{-1}$ was measured at the bottom of the metalimnion. At that time, the total metalimnetic phytoplankton biomass (TPB) was the highest of the season (Fig. 5A). The dominant phytoplankton taxa were chrysophytes (especially *Dinobryon* and *Synura*), which represented 98% of the biomass (Fig. 5B). Although chrysophytes reached a seasonal peak in August 2001 at $\sim 6,500 \text{ mg m}^{-3}$, bacterial abundance was relatively stable from mid-June to August (Fig. 5A). From August through September, both chlorophyll and chrysophytean biomass decreased, reaching $12 \mu\text{g Chl L}^{-1}$ (decrease of 56%) and 2293 mg m^{-3} (decrease of 65%), respectively.

In 2002, a different pattern was observed. The TPB was five times lower than that in 2001 (Fig. 5C), and two taxa dominated the metalimnion, dinoflagellates and chrysophytes, representing 50% and 38% of the biomass, respectively (Fig. 5D). From June to July 2002, bacterial abundance plummeted (loss of 72%), whereas phytoplankton biomass increased by 67% (Fig. 5C). A sharp decrease in TPB was observed mid-July (Fig. 5C,D) when dinoflagellates and chrysophytes biomasses declined by 85% and 50%. Then, chrysophytes became dominant (Fig. 5D), although bacterial biomass remained low (Fig. 5C). During the entire 2002 ice-free season, the relationship between metalimnetic chlorophyll and phytoplankton biomass was weak ($r^2 = 0.2$, $p = 0.361$). Metalimnetic chlorophyll was strongly related

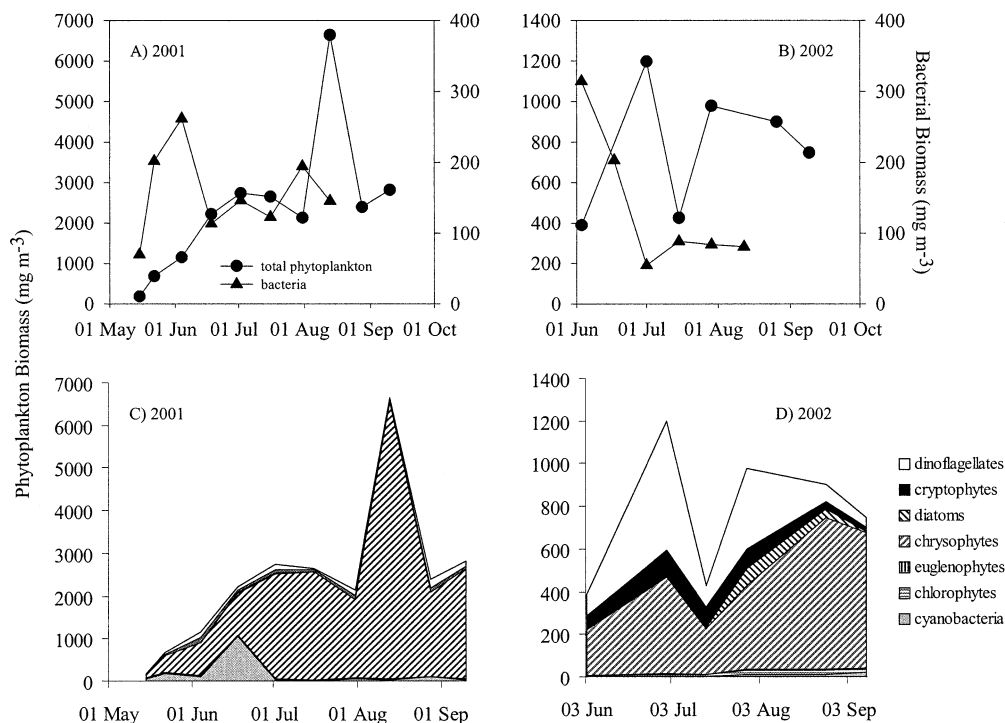


Fig. 5. (A) Temporal evolution of total phytoplanktonic (black circles) and bacterial (black triangles) biomasses in 2001 (A) and 2002 (C). Species composition of the phytoplankton biomass in Lake 658 in 2001 (B) and 2002 (D).

to chrysophyte biomass ($r^2 = 0.93$, $p = 0.002$), but it was unrelated to dinoflagellates ($r^2 = 0.05$, $p = 0.664$).

During winter, the water column on March 2 was dominated by chrysophytes, with well-identified peaks at depths of 2 and 9 m (Fig. 2C). The entire water column was oxygenated ($2 \text{ mg L}^{-1} < [\text{O}_2] < 11.58 \text{ mg L}^{-1}$). However, a decrease in oxygen concentration was observed at a depth of 9 m (Fig. 2C). DGM concentrations and TPB were well correlated ($r^2 = 0.68$, $p = 0.004$).

Microbiology of the depth profile in Lake 658—The dominant actively metabolizing bacteria in the 5.5 and 12 m depth in September 2001 were identified by their 16s rRNA sequences (Ramos et al. 2000). Fifty-four clones representing the community at 5.5 m depth were divided to 28 RFLP groups, and 49 clones representing the community at 12 m were divided to 27 RFLP groups. Shannon-Weaver diversity indices calculated as described by Barkay (1987) using the RFLP groups as taxonomic units indicated similar diversity of actively metabolizing bacteria at 5.5 and 12 m, with indices of 1.317 and 1.371 (unitless), respectively. Sequence analysis of 16s rRNA from each RFLP group and similarity searches against available databases showed dominance of strains belonging to the orders Planctomyces, whose members are facultative aerobic chemoorganotrophs found in oligotrophic freshwater and marine habitats (Staley et al. 1999), and the β -Proteobacteria. In addition, representatives of two RFLP groups from the anoxic water at 12 m were most closely related to the sulfate-reducing δ -proteobacterium *Desulfococcus multivorans*, and representatives of two RFLP groups from 5.5 m, located at the bottom of the metalimnion, were most closely related to the methanotrophic genus *Methylocaldum* spp.

Transcripts of the *merA* gene, which are indicative of the expression of genes encoding the mercuric reductase enzyme, were not detected in the RNA extracts that were obtained at either 5.5 or 12 m. Both 16s rRNA and *glnA* mRNA transcripts were present in these RNA extracts, as indicated by PCR products of the expected size obtained with primers specific to these targets. Thus, *merA* transcripts, if present, were below the detection limit of our method. At present, our PCR method can detect 100 *merA* transcripts per nanogram of community RNA (Yagi, pers. comm.). Because 1 ng of bacterial RNA represents $\sim 2 \times 10^4$ actively growing cells and *merA*-expressing cells contain 10^0 – 10^2 copies of *merA* transcripts (Yagi and Barkay unpubl. data), the failure to detect *merA* transcripts in Lake 658 depth samples indicates that <1 in 10^4 cells in the sampled biomass was actively expressing *mer* operon functions.

Discussion

Surface total DGM production—DGM production was mainly photomediated near the air-water interface, both in wetlands and in the pelagic zone (Fig. 1A–E). Also, in pelagic waters, this production was not controlled by the presence of living or dead particles, as shown by the filtration (porosity $0.1 \mu\text{m}$) experiment (Fig. 1A,B). Wetland water had faster DGM formation rates and DOC levels twice as high as those in lake water. Indeed, Hg reduction is thought to be

triggered by DOC through its photoexcitation. High DOC levels could therefore increase the concentration of Hg photoreductants at the air-water interface. However, DGM photoproduction yields were higher in surface lake water, which suggests that, even if reduction occurred faster in the wetland, potentially as a result of high concentrations of photoproduced reductants, the pool of photoreducible Hg was smaller, likely because of Hg binding by DOC.

Production of DGM at depth—Several abiotic or biotic Hg reduction processes can possibly explain the occurrence of reduced Hg maxima within the water column of lakes. First, humic substances can enhance abiotic Hg(0) production (Alberts et al. 1974). Further studies have shown that this reaction happens at low oxygen concentrations, in the absence of chloride, and is enhanced in the presence of light (Allard and Arsenie 1991). Both studies reported low production rates. In the case of our study, neither the bottom of the metalimnion in 2001 nor the metalimnion in 2002 were under anoxic conditions (Fig. 4A,B), and peaks in both 2001 and in 2002 were located between 1% and 5% of the surface incident light. Moreover, the daily and seasonal variations in the peaks do not suggest a role of humic substances. Second, Peretyazhko (2002) described Hg redox-cycle dynamics at the oxic-anoxic interface of lakes, invoking the coupling of the Hg(II)/Hg(0) and Fe(III)/Fe(II) redox cycles in the abiotic production of Hg(0) at the interface between the metalimnion and the anoxic hypolimnion. Others have further studied the ability of iron to directly reduce Hg (Charlet et al. 2002; O'Loughlin et al. 2003). Both studies concluded that strictly anoxic conditions were required to efficiently trigger Hg reduction to Hg(0) in the dark. In our study, anoxia was not encountered above a depth of 9 m in 2001, and in 2002 the sediment-water interface was barely anoxic at the time we performed our profiles. Moreover, reduced iron [Fe(II)] was not detectable at a depth of 5.5 m in August 2001 (Chadwick and Hurley pers. comm.), but it was present in mid-September. Those data are opposite to what we would expect if Fe(II) was involved in Hg(II) reduction. DGM peaks under the ice were not located at an oxic-anoxic interface, and such a mechanism could not explain the decrease of DGM concentrations we observed at night in 2001. The reduction of Hg(II) by Fe(II) is possible in oxic environments, when photoreduction of Fe(III) to Fe(II) occurs—that is, usually near the air-water interface (Zhang et al. 2001). Light intensity was not energetic enough to efficiently trigger a Fe(III)-induced DGM photoproduction at depths of 3.5 or 5.5 m. Therefore, it is unlikely that a coupling between Hg and Fe redox cycles is responsible for the production of DGM in the metalimnion of Lake 658. Third, Siciliano et al. (2002) correlated the microbial reductase activity with DGM concentrations within the water column of a mesotrophic lake located in Ontario (Canada). We tested the activity of the bacterial mercuric reductase by the presence of the *merA* mRNA transcript. Our results indicate that, if present, <1 in 10^4 cells express *merA* in the water column of Lake 658 at the depth where peak DGM concentrations were observed. Although we cannot exclude the bacterial reduction of Hg in the formation of DGM, because the half-life of *mer* transcripts is measured in minutes (Gambill and

Summers 1992), mercury reduction and *mer* operons were never described in the bacterial species that dominated the active communities at the depth where DGM accumulated in Lake 658 in September 2001. Furthermore, in 2002, the highest DGM concentrations in the season (01 July 2002) were recorded when the bacterial biomass was the lowest (Fig. 5C). We therefore conclude that microbial mercuric reductase activity could not explain the DGM production patterns in Lake 658 depth profiles. Finally, the production of DGM in the metalimnion of Lake 658 during the stratified period and the patterns observed under the ice may be, directly or indirectly, a result of phytoplankton activity. Indeed in 2001, DGM concentrations decreased sixfold at night, which suggests a link with phytoplankton photosynthetic activity. This decrease in DGM concentrations may have resulted from DGM diffusing in adjacent water layers, although the diffusion of gases in the metalimnion is hampered by density gradients. Alternately, abiotic or biotic oxidation may be involved. Because dark abiotic oxidation is generally slow, bacterial oxidation is more likely (Smith et al. 1998).

In 2002, we did not observe this decrease at night; however, DGM concentrations were strongly correlated to total phytoplankton biomass, especially to that of chrysophytes and dinoflagellates ($[DGM] = 0.0175 \exp^{(0.0063[TPB])}$, $r^2 = 0.95$, $p = 0.0032$). DGM concentrations in the metalimnion decreased below our detection limit when the phytoplankton biomass declined by 75% on 15 July (Fig. 2D,E), then rose again in August when phytoplankton biomass increased.

Role of mixotrophic algae in DGM formation—Phytoplankton blooms that occur in the metalimnion of oligotrophic or oligomesotrophic lakes are often mixotrophic (Bird and Kalff 1989). These organisms can preferentially use either DOC or bacteria as a source of carbon rather than fixing inorganic carbon when environmental conditions are not favorable (i.e., low light levels, low nutrients, or a lack of growth factors; Bird and Kalff 1986; Caron et al. 1993; Sanders et al. 2001). Despite the lack of information on the metabolic pathways that lead autotrophic organisms to use organic carbon or to switch from total autotrophy to partial or obligate heterotrophy, this phenomenon has been commonly observed, including recently in lakes of the Experimental Lakes Area (Findlay et al. 2001). The dominant dinoflagellates and chrysophytes in Lake 658 in 2001 and 2002 were potential mixotrophs showing a strong bacterivorous ability when required (Bird and Kalff 1986; Berninger et al. 1992; Caron et al. 1993).

This major characteristic of phytoplankton thriving in oligotrophic lakes could explain the different night-versus-day DGM patterns observed in 2001 and 2002, if DGM production is linked to carbon acquisition by algae. In 2001, we consider that the metalimnetic bloom was mainly formed of autotrophic organisms, because (1) we observed a sixfold decrease in DGM at night, consistent with a decrease in photosynthetic activity and carbon acquisition; (2) increases in phytoplankton biomass in August did not result in a decline in bacterial abundance, as would have resulted from predator-prey interactions; and (3) there was a significant relationship between chlorophyll *a* and phytoplankton biomass.

In 2002, we consider that the bloom was partly heterotrophic, because (1) there was no change in DGM concentrations between day versus night, which is consistent with continuous daily carbon assimilation by heterotrophs; (2) the 75% decrease in bacterial biomass in mid-July 2002 (Fig. 5C) may have resulted from predator-prey interactions; (3) during the whole spring and summer 2002, a weak relationship existed between total phytoplankton biomass and the chlorophyll concentration in the metalimnion ($r^2 = 0.2$, $p = 0.36$). This relationship was not observed for dinoflagellates ($r^2 = 0.05$, $p = 0.66$) but was very strong for chrysophytes ($r^2 = 0.93$, $p = 0.02$). We therefore postulate that dinoflagellates were mainly heterotrophic in 2002, using bacterivory to assimilate carbon. The status of chrysophytes is harder to determine, because the presence of pigment does not exclude phagotrophy (Tuchman 1996). However, that chrysophytes increased in biomass while bacteria did not decline and the strong correlation between chlorophyll and chrysophytes suggest autotrophy.

Effect of DCMU on DGM formation—The in situ experiments done at a depth of 3.5 m in August 2002 showed that DGM formation only occurred in samples unfiltered and unamended with DCMU. DGM production was not observed in treatments that were kept in the dark, when water was filtered (1 μm porosity) or when an inhibitor of photosynthesis (DCMU) was added (Fig. 5A,B). Rather, a decrease was observed in the latter cases. We attribute this decrease to oxidation reactions that occur in the dark (Amyot 1997b). As was stated above, this decrease supports our hypothesis that phytoplankton influence DGM production. At the time of the experiment, the phytoplankton community was dominated by chrysophytes (up to 79%) that we believe to be autotrophic (as opposed to dinoflagellates earlier in the season) and thus metabolically influenced by darkness or an inhibitor of photosynthesis. It is therefore likely that the inhibition of photosynthesis during dark conditions or after the addition of DCMU strongly decreased the effect of phytoplankton on DGM production.

DGM under the ice—It is unlikely that, under ice, phytoplankton use photosynthesis to fix inorganic carbon in the water column, especially at a depth of 9 m. Several alternatives strategies can be used by algae to survive over the winter period. They can either develop resistance forms such as spores or cysts or use heterotrophy to survive (Berninger et al. 1992; McKnight et al. 2001). It is not surprising in this case to observe a chrysophytes peak at depths of 2 and 9 m coinciding with a decrease in the oxygen concentration, suggesting an area with increased respiration. The production of DGM, as shown by the peaks present at depths of 2 and 9 m, is likely the result of the activity of these chrysophyte communities, because both were well correlated ($r^2 = 0.68$, $p = 0.004$).

Hypolimnetic DGM concentrations—Very little is known about the production of DGM in anoxic zones. Bacterial mercuric reductase may be active under these conditions but with very low efficiencies (Golding et al. 2002; Schaefer et al. 2002). Also, humic substances may induce DGM pro-

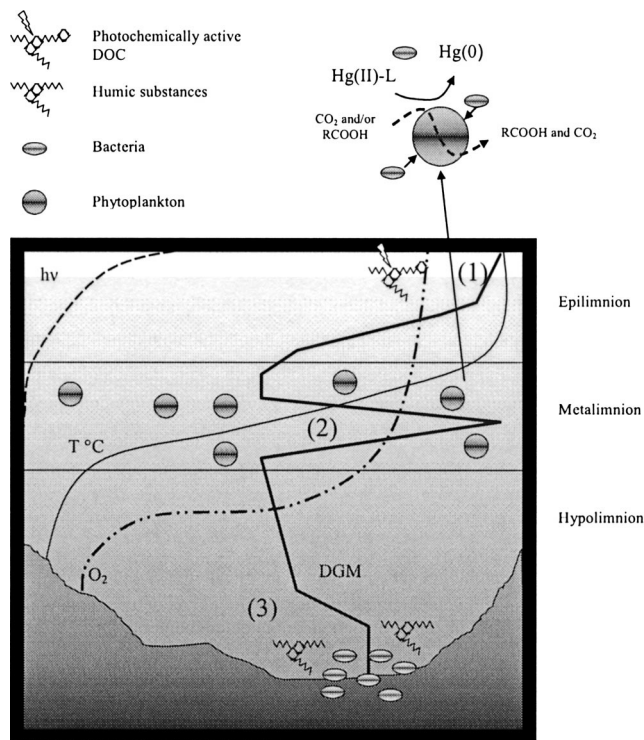


Fig. 6. Scheme summarizing DGM distribution in the water column of Lake 658.

duction (Allard and Arsenie 1991). Because *merA* transcripts were not detected in the 12-m sample that was collected in September 2001, we believe that DGM production at the bottom of the water column is either the result of an abiotic reaction involving humic substances or is carried out by microorganisms that reduce Hg by pathways that remain to be determined.

Integrated overview of DGM production—We propose a schematic overview of the distribution of DGM concentrations within the water column of Lake 658 (Fig. 6). First, at the surface (1), DGM production is photochemically mediated, because no DGM production was observed in the dark and the removal of organisms $>0.1 \mu\text{m}$ does not influence reduction rates or hourly photoproduction levels. In the metalimnion, (Fig. 6; eq. 2), DGM production is strongly linked to phytoplankton dynamics. The formation of Hg(0) occurs during phytoplankton blooms and not in a continuous manner. Rather than a linear increase, DGM levels increases exponentially with phytoplanktonic biomass, which suggests that the production of DGM is not directly linked to photosynthesis but rather is dependent on either the generation of reductants inside the cells or on the excretion of those reductants into the surrounding water. Indeed, autotrophic and mixotrophic algae are expected to excrete an excess of organic compounds as byproducts of light reactions when photosynthesis is not inhibited by the ingestion of organic carbon (Tuchman 1996). Finally, at the sediment-water interface (Fig. 6; eq. 3), DGM production likely results from chemical reactions involving humic substances in anoxic environment or as-yet unidentified microbial activities.

Many oligomesotrophic boreal lakes have mixotrophic phytoplankton communities that can thrive when nutrients or light are low or absent. Therefore, phytoplankton can, even if conditions are not favorable for photosynthesis, influence Hg redox cycling by generating Hg(0). This is an important component of the Hg cycle within the water column of lakes because, under its reduced form, Hg may interact differently with particles or methylating bacteria.

Mechanisms by which Hg is reduced or demethylated to Hg(0) in low-light, pristine environments are still unknown, and further investigations should explore the intricate pathways involved in photosynthesis, carbon fixation, and carbohydrate formation, to better characterize the conditions required for the production of dissolved gaseous mercury.

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