Bioaccumulation of newly deposited mercury by fish and invertebrates: an enclosure study using stable mercury isotopes

Michael J. Paterson, Paul J. Blanchfield, Cheryl Podemski, Holger H. Hintelmann, Cynthia C. Gilmour, Reed Harris, Nives Ogrinc, John W.M. Rudd, and Ken A. Sandilands

Abstract: Enriched stable mercury (Hg) isotopes were added to four 10 m diameter enclosures in Lake 239 at the Experimental Lakes Area to increase inorganic Hg loading. Our main objectives were to (*i*) follow low-level additions (spikes) of isotope-enriched Hg through the biogeochemical cycle and into the food web and (*ii*) determine the relative contribution of newly deposited Hg to methyl Hg (MeHg) accumulation by fish and other biota. The experiment ran for two summers (2000, 2001), with different enriched Hg isotopes being added each year. Within 1 month of beginning additions in 2000, spike Hg was detected in water, zooplankton, and benthic invertebrates as MeHg, and in fish as total Hg (THg; the sum of inorganic and organic Hg). In 2001, concentrations in water of inorganic spike Hg added in 2000 were near detection limits, but concentrations of 2000 spike MeHg in water and biota remained unchanged or greater. Despite comparatively large increases in inorganic Hg loading, accumulation of ambient, non-spike MeHg predominated in all organisms, and spike MeHg never comprised more than 15%, even after 1 year. Our results suggest that changes in Hg loading will affect MeHg concentrations in fish and other biota, but that steady state may not be achieved for at least 10–30 years under conditions similar to our enclosures.

Résumé : Nous avons ajouté des isotopes stables enrichis de mercure (Hg) à quatre enclos de 10 m de diamètre au lac 239 dans la Région des lacs expérimentaux afin d'augmenter la charge de Hg inorganique. Nos objectifs principaux étaient (*i*) de suivre des additions de faible niveau (pics) d'Hg enrichi d'isotopes à travers le cycle biogéochimique et dans le réseau alimentaire et (*ii*) de déterminer la contribution relative du Hg nouvellement déposé à l'accumulation de méthyl Hg (MeHg) par les poissons et les autres organismes. L'expérience s'est poursuivie pendant deux étés (2000, 2001) avec différents isotopes enrichis de Hg ajoutés chaque année. En moins de 1 mois après le début des additions en 2000, un pic a pu être détecté sous forme de MeHg dans l'eau, le zooplancton et les invertébrés benthiques et sous forme d'Hg total (THg, la somme du Hg inorganique et organique) chez les poissons. En 2001, les concentrations dans l'eau du pic de Hg inorganique ajouté en 2000 étaient à la limite de la détection, mais les concentrations du pic de MeHg de 2000 dans l'eau et les organismes étaient les mêmes ou avaient augmenté. Malgré des augmentations relativement importantes de la charge de Hg inorganique, l'accumulation du MeHg ambiant non relié au pic prédomine chez tous les organismes et le MeHg du pic ne représente jamais plus de 15 % même après 1 an. Nos résultats indiquent que des changements de la charge de Hg affectent les concentrations de MeHg chez les poissons et les autres organismes, mais que l'équilibre ne sera pas atteint avant 10–30 ans sous des conditions comparables à celles de nos enclos.

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Introduction

Concentrations of methyl mercury (MeHg) are elevated in fish from many lakes in Canada and the United States. This has resulted in the closure of fisheries and the issuance of consumption advisories in many provinces and states. Even lakes located far from point source inputs of Hg often have high concentrations of MeHg in fish, and there is increasing

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M.J. Paterson,[1] P.J. Blanchfield, C. Podemski, J.W.M. Rudd,[2] and K.A. Sandilands. Fisheries and Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada.
H.H. Hintelmann and N. Ogrinc.[3] Trent University, Department of Chemistry, 1600 West-Bank Drive, Peterborough, ON K9J 7B, Canada.
C.C. Gilmour. Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD 21037, USA.
R. Harris. Tetra Tech Inc., 180 Forestwood Drive, Oakville, ON L6J 4E6, Canada.

[1]Corresponding author (e-mail: patersonm@dfo-mpo.gc.ca).
[2]Present address: R & K Research Inc., 675 Mt. Belcher Heights, Salt Spring Island, BC V8K 2J3, Canada.
[3]Present address: Jozef Stefan Institute, Department of Environmental Sciences, Jamova 39, 1000 Ljubljana, Slovenia.

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recognition of the importance of atmospheric Hg loading (Jackson 1997; Fitzgerald et al. 1998). A large proportion of atmospheric Hg is generated from anthropogenic emissions, particularly from coal-fired power plants (Mason et al. 1994). As a result, reductions of anthropogenic Hg emissions to the atmosphere of 50% or more have been proposed in both Canada and the United States, with the aim of reducing MeHg concentrations in fish (North American Implementation Task Force on Mercury 2000).

Once deposited in a lake, inorganic Hg that predominates in precipitation must be converted to MeHg before transfer to fish. Although it is widely agreed that levels of atmospheric Hg deposition are probably linked to levels of MeHg in fish (e.g., Johansson et al. 1991; Downs et al. 1998), this relationship has been difficult to demonstrate. In part, this is because the amount of stored Hg in soils and sediments of terrestrial and aquatic ecosystems is hundreds to thousands of times greater than annual deposition. As a result, it is not clear whether most of the Hg methylated in any given year was deposited during that year or during many previous years. Large differences in factors affecting Hg methylation and MeHg availability to the food web further obscure the relationship between Hg deposition and Hg accumulation by fish (Wiener et al. 2003).

An understanding of the relative contribution of recently deposited Hg to Hg accumulation by fish and other biota is required to predict whether changes in fish Hg will occur slowly or rapidly following changes in Hg emissions. If MeHg accumulated by fish and other biota is primarily derived from newly deposited Hg, then concentrations in fish should change rapidly following changes in Hg emissions. Alternatively, if much of the Hg in fish is derived from Hg deposited in past years, MeHg concentrations in fish will change more slowly with changes in atmospheric Hg deposition. For example, Hrabik and Watras (2002) observed simultaneous declines in Hg in fish and deposition to Little Rock Lake, a seepage lake in Wisconsin. From this, they inferred that newly deposited Hg predominantly contributed to the Hg accumulated by fish in this lake.

Our study was undertaken using large enclosures in Lake 239 (L239) at the Experimental Lakes Area (ELA) in northwestern Ontario and was completed in advance of a whole-lake Hg isotope addition experiment called METAALICUS (Mercury Experiment To Assess Atmospheric Loading in Canada and the United States). The METAALICUS study is exploring the effect of changes in inorganic Hg deposition rates on Hg accumulation by fish and their associated food web. In both our enclosure study and the whole-lake experiment, recently deposited Hg is being distinguished from old Hg by adding Hg that is enriched with certain stable isotopes. Stable isotopes of Hg have rarely been used in an ecosystem context or to follow patterns of food web transfer (e.g., Hintelmann et al. 2002; Pickhardt et al. 2002, 2005). Our objectives in the enclosure study were to (*i*) assess the use of low-level additions of isotope-enriched Hg to follow Hg through the biogeochemical cycle and into the food web; (*ii*) determine the timing and relative contribution of newly deposited Hg to Hg accumulation by fish and other biota; and (*iii*) compare results from enclosures receiving isotopically enriched Hg in a single annual dose versus multiple doses over the summer.

Table 1. Percent contribution of different stable isotopes to the Hg spikes added to the L239 enclosures in 2000 and 2001.

Isotope	2000	2001
^{196}Hg	<0.02	<0.10
^{198}Hg	0.13	<0.10
^{199}Hg	0.99	0.01
^{200}Hg	96.41	0.30
^{201}Hg	1.46	0.10
^{202}Hg	0.10	99.20
^{204}Hg	0.10	<0.05

Materials and methods

Experimental design

Four large enclosures (10 m diameter × approximately 1.5–2 m deep) open to both lake sediments and the atmosphere were installed in L239 at the ELA (49°39′N, 93°43′W) on 6–7 June 2000. Enclosure walls were made of woven polyethylene supported by a foam collar at the surface and anchored to the bottom with two rings of sandbags placed on a skirt sewn to the enclosure wall. Such enclosures are not completely water tight. Orihel (2005) estimated water losses from identical enclosures installed in nearby Lake 240 (L240) using additions of ^{3}H. She found average water leakage of 6.6% between June and September of 2002.

Our experiment started in June 2000 and continued through to September 2001. A spike enriched with different isotopes of Hg was added each summer. In 2000, enclosures were amended with a spike solution enriched with ^{200}Hg and in 2001 with ^{202}Hg (the exact isotopic composition of each spike is listed in Table 1). Because neither spike solution consisted entirely of a single isotope, we determined the excess concentration of the major spike isotope in each sample and mathematically corrected the final concentration of each spike, taking into account the concentrations of minor isotopes in the spike solutions (Hintelmann and Ogrinc 2003). This allowed us to separate naturally occurring background (or ambient) Hg from the isotopically enriched spike Hg. Below, we refer to the two spikes as the 2000 spike and the 2001 spike.

We increased annual wet Hg loading by adding 30 $\mu g \cdot m^{-2}$ of spike Hg to each enclosure. Long-term annual wet deposition of Hg at ELA is approximately 4 $\mu g \cdot m^{-2}$ and was between 7.1 and 7.9 $\mu g \cdot m^{-2}$ from 1998 to 2001 (St. Louis et al. 2004). Additions were made according to two different regimes. Two enclosures (E1 and E2) each received a single 30 $\mu g \cdot m^{-2}$ addition on 15 June 2000 (day 1) and 13 July 2001 (day 395). In each summer, additions were made to E3 and E4 every 2 weeks for 5 weeks with lower doses (6 $\mu g \cdot m^{-2}$) so that the cumulative additional loading in all enclosures was the same.

The isotope stock solutions were made by dissolving the isotope-enriched Hg (as Hg^0) in dilute HCl. Prior to addition, an aliquot of $HgCl_2$ solution was added to 500 mL of L239 water and allowed to equilibrate for at least 4 h. The spike solution was then added at dusk from a raft in the cen-

ter of each enclosure and mixed in using a paddle in 2000 and a small electric trolling motor in 2001.

Water and sediment sampling

Samples for the determination of water chemistry and dissolved and particulate Hg were collected from a raft held in the center of each enclosure. Samples were collected at least biweekly in 2000, whereas in 2001 a diminished sampling schedule was used to follow only longer term changes in Hg accumulation. Water samples for Hg analysis were pumped from a depth of 0.75 m and filtered through a pre-ashed quartz fiber filter (Whatman QM-A; 0.7 μm nominal pore size) housed in an in-line Teflon cartridge system. Clean hand – dirty hand protocols were used throughout (Horowitz et al. 1994). Samples were collected and stored in 250 mL acid-cleaned Teflon bottles. For particulate Hg, an additional 500–1000 mL of water was filtered to obtain sufficient mass for analysis.

Sediments were collected monthly in 2000 and twice in 2001 from the enclosure rafts using 4.8 cm diameter polyvinyl chloride (PVC) core tubes mounted on a PVC tube fitted with a clamp to seal the headspace. On each sampling date, three cores were collected from each enclosure from randomly selected locations. Great care was taken to minimize sediment disturbance during sampling. Cores were sectioned and preserved by freezing within hours of collection.

Invertebrate sampling

Zooplankton were collected weekly in 2000 using horizontal sweeps of a 150 μm net attached to a wooden pole. In 2001, zooplankton were sampled only once in September. Samples were prepared for MeHg analysis using methods described in Paterson et al. (1998). Zooplankton consisted predominantly of Cladocera (*Bosmina longirostris*, *Diaphanosoma birgei*, *Daphnia mendota*), cyclopoid copepods (*Mesocyclops edax*, *Tropocyclops extensus*), and calanoid copepods (*Diaptomus minutus*). Benthic invertebrates were collected monthly in 2000 using an Ekman dredge, with care being taken to minimize the amount of sediment released into the water column. Dredge contents were washed through a sieve outside the enclosures. In 2001, macroinvertebrates were collected only in September using a d-frame dipnet. After collection, invertebrates were removed from debris while alive, sorted to the lowest practical taxonomic level, and frozen. Samples were freeze-dried, ground to a powder using acid-washed glass rods, and weighed to the nearest 0.1 μg. They were then transferred to a 5 mL conical bottom Teflon vial for digestion.

Fish stocking and sampling

Immediately after enclosure installation in 2000, baited minnow traps and small-mesh gill nets were used in an attempt to remove all fish from the enclosures. Finescale dace (*Phoxinus neogaeus*) were then purchased from a local baitfish supplier, and 30 were added to each enclosure on 14 June 2000. At initial stocking, 10 finescale dace were kept as a reference sample. The density of fish in each enclosure (0.35 fish·m^{-2}) approximated densities used in other enclosure experiments (Bodaly and Fudge 1999) and in shallow lakes at the ELA (Eddy 2000). In June 2001, as many fish as possible were again removed from the enclosures using minnow traps and gillnets. On 18 July (day 398), we stocked each enclosure with 15 finescale dace collected from ELA Lake 114. To differentiate newly added fish, all stocked finescale dace received a clip on the upper caudal fin.

In 2000, fish were collected monthly using overnight sets of minnow traps and then killed, measured (fork length), weighed, and frozen. In 2001, enclosures were sampled for fish only in September. There was large, unexplained mortality of finescale dace after the first sampling period in E2 and E3 in 2000 and in E1 and E2 in 2001. As a result, data presented for 2000 include only fish collected from E1 and E4, and data for 2001 include only fish from E3 and E4. At least four finescale dace were captured on all dates for which data are presented. In addition to finescale dace, a few small yellow perch (*Perca flavescens*; mostly young of the year and age 1+) were also captured (n = 1–7 from each enclosure on each sampling date). These fish naturally inhabit L239 and presumably were trapped when the enclosure walls were dropped in June 2000.

After collection, stomachs were removed from larger fish and preserved in 95% ethanol. Approximately 0.2 g of muscle tissue was removed from each fish, placed into a clean glass scintillation vial, and frozen prior to analysis for total Hg (THg; the sum of inorganic and organic Hg). THg was measured because generally >90% of the Hg in fish muscle is MeHg (Bloom 1992; Turner and Rudd 1983). We analysed the stomach contents of finescale dace (n = 44) and yellow perch (n = 7) that were captured in enclosures E1 and E4 in 2000. Each stomach was cut open and all items were counted individually and identified to order using a compound microscope. Organisms from the same order were grouped and oven-dried to determine their contribution by weight to fish diet.

Analytical methods

Water chemistry other than Hg was analysed using methods in Stainton et al. (1977). THg in water samples was determined after oxidation of 50 mL samples by adding 0.25 mL of BrCl. The method for digesting particles collected on filters has been modified from Morrison and Watras (1999). Filters were fitted into 30 mL Teflon vials and 10 mL of Milli-Q water were added. Particles were digested by adding 0.1 mL concentrated HCl and 0.1 mL BrCl (0.2 mol·L^{-1}). Fish samples were weighed into 25 mL glass vials and digested by adding 10 mL of a HNO_3–H_2SO_4 (7:3) mixture. Samples were heated to 80 °C until the formation of brown NO_x gases had ceased. All samples were then diluted and analyzed. Prior to digestions, water and particulate samples received 20 μL and fish samples 500 μL of a $^{201}Hg(II)$ standard solution (2.3 ng·mL^{-1}) to serve as an internal standard. Ionic Hg in the water and particulate digests was reduced using stannous chloride and the generated Hg^0 purged onto gold traps, which were heated to release the Hg. THg in fish was quantified using an on-line flow injection system described in Hintelmann and Ogrinc (2003). Measurements were performed by inductively coupled plasma mass spectrometry (ICP-MS, Micromass Platform). Sediment analysis was performed on thawed, homogenized, wet samples. Digestion methods for THg and distillation methods for MeHg in sediments are described in Gilmour et al.

Table 2. Average water chemistry in the study enclosures in 2000.

Parameter	E1	E2	E3	E4	Lake 239*
pH	7.1 (0.1)	7.2 (0.1)	7.2 (0.1)	7.3 (0.1)	7.0 (0.1)
Total phosphorus ($\mu g \cdot L^{-1}$)	7.2 (3.7)	9.2 (3.0)	6.4 (1.9)	8.3 (2.0)	8.1 (5.4)
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	1.6 (1.0)	1.3 (0.8)	1.5 (1.2)	1.2 (0.7)	2.6 (0.7)
Suspended carbon ($\mu g \cdot L^{-1}$)	558 (222)	611 (226)	492 (128)	493 (136)	750 (177)
DOC ($mg \cdot L^{-1}$)	6.9 (0.3)	7.0 (0.4)	6.5 (0.3)	6.4 (0.3)	8.1 (0.9)
Zooplankton biomass (μg dry weight$\cdot L^{-1}$)	84.4 (99.1)	50.6 (73.5)	57.0 (59.4)	55.6 (79.0)	16.1 (7.7)

Note: Numbers in brackets are 1 standard deviation. DOC, dissolved organic carbon.
*Parameter values for Lake 239 are for the epilimnion at centre-buoy, except for zooplankton, which is a whole-water-column estimate.

Table 3. Average, time-weighted ambient Hg concentrations in the enclosures in 2000 and 2001 (all enclosures).

	2000	2001
Water		
Unfiltered THg ($ng \cdot L^{-1}$)	1.6 (0.4)	1.1 (0.1)
Unfiltered MeHg ($ng \cdot L^{-1}$)	0.11 (0.02)	0.06 (0.01)
Sediment (0–2 cm)		
THg ($ng \cdot g^{-1}$ dw)	17.6 (4.4)	15.6 (5.8)
MeHg ($ng \cdot g^{-1}$ dw)	0.54 (0.21)	0.57(0.13)
Invertebrates		
Zooplankton MeHg ($ng \cdot g^{-1}$ dw)	108 (26)	58 (20)
Ceratopogonidae MeHg ($ng \cdot g^{-1}$ dw)	81 (32)	—
Chironomidae MeHg ($ng \cdot g^{-1}$ dw)	47 (17)	38 (14)
Ephemeroptera MeHg ($ng \cdot g^{-1}$ dw)	41 (16)	44 (6)
Hydracarina MeHg ($ng \cdot g^{-1}$ dw)	220 (194)	53 (15)
Pisidiidae MeHg ($ng \cdot g^{-1}$ dw)	44 (60)	13 (4)
Fish		
Finescale dace THg (*Phoxinus neogaeus*; $ng \cdot g^{-1}$ ww)	108 (27)	174 (46)
Yellow perch THg (*Perca flavescens*; $ng \cdot g^{-1}$ ww)	76 (25)	109 (54)

Note: Numbers in brackets are 1 standard deviation. Ceratopogonidae were not encountered in the 2001 samples. THg, total Hg (the sum of inorganic and organic Hg); MeHg, methyl Hg; dw, dry weight; ww, wet weight.

(1998). All sediment analyses were undertaken using a Perkin Elmer Elan DRC ICP-MS.

MeHg determinations were slightly modified from procedures described in detail by Horvat et al. (1993), Bloom (1989), and Hintelmann and Ogrinc (2003). Water samples (50 mL) were distilled in a glass distillation apparatus consisting of two 50 mL glass tubes connected via an air-cooled glass distillation bridge (inner diameter 8 mm). Particles collected on filters were distilled in a similar fashion, except that 30 mL Teflon bottles were used instead of the glass distillation system. Filters were placed in the vial and then 10 mL of Milli-Q water and reagents were added. Extraction of MeHg from freeze-dried zooplankton and zoobenthos was accomplished by treating the samples with a 20% (*w*/*v*) KOH–MeOH solution at 50 °C over 8 h, following Bloom (1992). MeHg in sample distillates and alkaline leaching solutions was measured after aqueous phase ethylation using $NaBEt_4$ (Hintelmann and Ogrinc 2003). Volatile Hg species were purged and trapped onto Tenax, and MeHg was measured after thermodesorption and GC separation using ICP-MS detection. To correct for procedural recoveries, $Me^{201}HgCl$ additions of 20 and 50 pg were made to water and biota samples, respectively, prior to distillation or digestion.

Samples of similar weight of a certified reference material (DORM-2, National Research Council of Canada (NRC), Ottawa, Ontario; NBS-1566 oyster tissue, National Bureau of Standards) were submitted to the same procedures; measured THg and MeHg concentrations in the reference materials were not significantly different from certified values ($p < 0.05$).

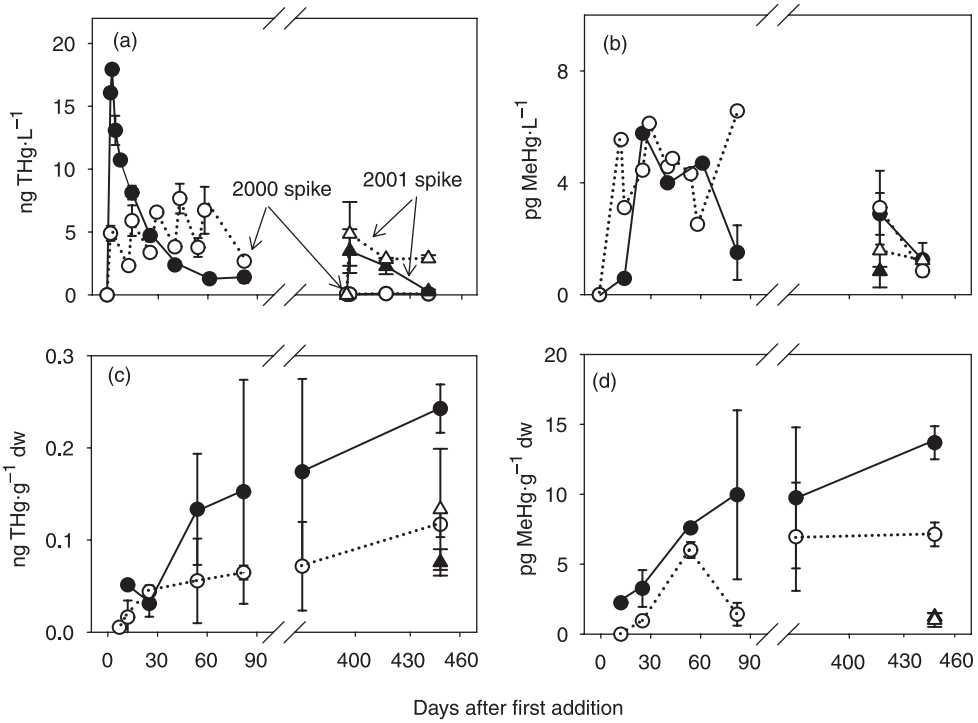
Terminology

In the discussion below, we use the following terms to distinguish the various forms of Hg in the enclosures. Methyl Hg (MeHg) refers to monomethyl Hg. Ambient THg or ambient MeHg refers to background THg or MeHg, respectively, not associated with the isotope-enriched Hg spikes. The 2000 spike refers to the Hg spike enriched with ^{200}Hg added in 2000, whereas the 2001 spike refers to the Hg spike enriched with ^{202}Hg added in 2001.

Spike/ambient ratios

We used the ratio of spike and ambient Hg as a measure of the amount by which THg or MeHg concentrations were changed by the addition of isotopically enriched Hg. For example, a spike/ambient ratio of 1.0 indicates that concentra-

Fig. 1. Changes in concentrations of (*a*) 2000 and 2001 spike total mercury (THg, the sum of inorganic and organic Hg) in unfiltered water samples (±1 standard error of the mean (SEM)); (*b*) 2000 and 2001 spike methyl mercury (MeHg) in unfiltered water samples (±1 SEM); (*c*) 2000 and 2001 spike THg in 0–2 cm sediments (±1 SEM); and (*d*) 2000 and 2001 spike MeHg in 0–2 cm sediments (±1 SEM). Standard errors reflect variability among enclosures receiving either a single Hg isotope addition (E1, E2) or multiple additions (E3, E4). Data for 18 July 2001 (day 396) are for water samples filtered through a quartz fiber filter. For MeHg, data points below detection were not included in averages. Symbols are as follows: 2000 spike, single addition (solid circles, solid line); 2000 spike, multiple additions (open circles, dotted line); 2001 spike, single addition (solid triangles, solid line); 2001 spike, multiple additions (open triangles, dotted line).



tions of spike and ambient Hg are equal and that the sum of spike and ambient Hg concentrations has increased by two times as compared with ambient Hg alone. The use of spike/ambient ratios facilitates comparisons of relative changes between different forms of Hg (MeHg, THg) and among different media and biotic groups (e.g., water, zooplankton, benthos, and fish). Spike/ambient ratios also help to normalize seasonal variations in overall Hg transfer and accumulation.

At the beginning of each year, finescale dace stocked in enclosures contained ambient Hg accumulated prior to their introduction. In 2000, initial ambient Hg concentrations were 86.8 ± 17.7 ng·g^{-1} wet weight (ww; mean ± 1 standard deviation) and in 2001, 178.8 ± 34.6 ng·g^{-1} ww. Most of this was presumably MeHg (Bloom 1992) that was probably retained for most of the 3-month duration of each summer period of the experiment. For example, the model of Trudel and Rasmussen (1997) indicates that a 3 g fish (similar to the size of finescale dace in the enclosures) depurates MeHg with a half-life of >250 days. Because we were interested in determining changes in the accumulation of spike versus ambient Hg while biota inhabited the enclosures, we calculated spike/ambient ratios for finescale dace using estimates of new ambient Hg accumulated during the experiment. This was done by subtracting the average muscle burden of ambient Hg in finescale dace collected on day 0 from the muscle burden of ambient Hg measured in fish on different sampling days. Muscle burdens of Hg were estimated as

$$(\mu g\ THg \cdot g^{-1}\ ww) \times (ww\ of\ muscle\ in\ each\ fish)$$

The weight of muscle (g) was assumed to be 49% of body weight (J. Van Wallegham, 501 University Crescent, Winnipeg, MB, Canada, R3T 2N6, personal communication, 2005). Spike/ambient ratios were then estimated as muscle burden of spike Hg divided by muscle burden of newly accumulated ambient Hg.

No such correction was necessary for invertebrate spike/ambient ratios because most of their population biomass (and associated MeHg) was accumulated during the experiment. Zooplankton have generation times of days to weeks in midsummer, and most macroinvertebrates at ELA are univoltine and undergo their greatest periods of growth during the summer months. No correction for ambient Hg in yellow perch was possible because no determinations of Hg burden were made at the experiment's initiation. As a result, we only consider spike/ambient ratios for the 2000 spike in yellow perch collected in September 2001. These perch had presumably accumulated most of their ambient Hg in the enclosures, because they had lived in them for more than 1 year.

Results

Water chemistry

Water chemistry was very similar among the enclosures, and most parameters changed minimally over the course of the experiment (Table 2). As is typical for lakes on the Canadian Shield, L239 is oligotrophic, circumneutral, and relatively high in dissolved organic carbon (DOC). Conditions within the enclosures were similar to the epilimnion of L239, except that concentrations of DOC, suspended carbon, and chlorophyll *a* were lower. In part, this reflects greater settling of particles in the more quiescent water column of the enclosures. DOC concentrations were lower because the enclosures were isolated from water exchange with the rest of the lake. This resulted in lower import of DOC in runoff from the watershed and, possibly, greater DOC loss from flocculation, sedimentation, and photobleaching. Water in the enclosures did not strongly stratify or become anoxic.

Ambient Hg concentrations

Concentrations of ambient THg and MeHg were generally low and typical for lakes on the Canadian Shield (Table 3). There were no obvious changes in concentrations of ambient Hg associated with the addition of the Hg spikes in 2000 or 2001. There was also no evidence that disturbance of lake sediments during enclosure installation or benthic sampling strongly affected ambient Hg concentrations in the water column or biota. Concentrations of unfiltered THg and MeHg from L239 were only measured in the enclosures, but were similar to those measured simultaneously in L240, which is immediately downstream of L239 (L240 THg = 1.37 ± 0.26 ng·L^{-1}; MeHg = 0.06 ± 0.03 ng·L^{-1}). Comparisons of unfiltered THg and MeHg collected within and outside of identical enclosures in a second experiment conducted in L240 in 2002 and 2003 revealed no statistically significant differences between the lake and enclosures (D. Orihel, 501 University Crescent, Winnipeg, MB, Canada, R3T 2N6, personal communication, 2006). Concentrations of unfiltered THg also did not increase in samples collected immediately after benthic sampling as compared with samples collected before sampling (paired *t* test, $p > 0.4$). In 2000, concentrations of ambient MeHg in L239 zooplankton collected within and outside of the enclosures were statistically indistinguishable (average time-weighted concentration in enclosures = 108 ± 26 ng·g^{-1} dry weight (dw); in L239 = 74 ± 23 ng·g^{-1} dw; paired *t* test, $p > 0.2$).

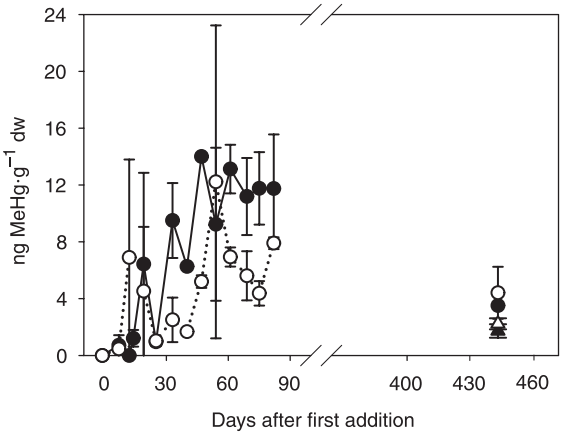
Where data were available, there were few consistent directional changes in concentrations of ambient THg or MeHg during the course of each summer. Exceptions were Ceratopogonidae, which increased in average ambient MeHg concentrations from 75 to 110 ng·g^{-1} dw, and finescale dace, which increased in average THg concentrations from 87 to 122 ng·g^{-1} ww between June and September 2000. Overall, concentrations of ambient THg and MeHg in water and invertebrates were lower in 2001 than in 2000.

Spike Hg and MeHg concentrations

Water and sediments

In enclosures receiving a single Hg addition (E1 and E2), concentrations of the added spikes as THg in water declined rapidly in both 2000 and 2001 (Fig. 1*a*). In enclosures receiving multiple Hg doses (E3 and E4) in 2000, concentrations of the spike increased immediately after each addition and then diminished to create a sawtooth pattern over time. This pattern was not discernable in 2001 because of less intensive sampling. In September 2000 (day 82), concentrations of spike THg in unfiltered water were similar in enclosures receiving single and multiple doses. In 2001, very little of the 2000 spike was still detectable in water (average concentrations <3%). Enclosures receiving multiple additions usually had higher concentrations of 2001 spike THg than enclosures receiving a single addition. The reason for this difference is uncertain.

Fig. 2. Changes in concentrations of the 2000 and 2001 spike methyl mercury (MeHg) in zooplankton (±1 SEM). Standard errors reflect variability among enclosures receiving either a single Hg isotope addition (E1, E2) or multiple additions (E3, E4). Symbols are as follows: 2000 spike, single addition (solid circles, solid line); 2000 spike, multiple additions (open circles, dotted line); 2001 spike, single addition (solid triangles); 2001 spike, multiple additions (open triangles).

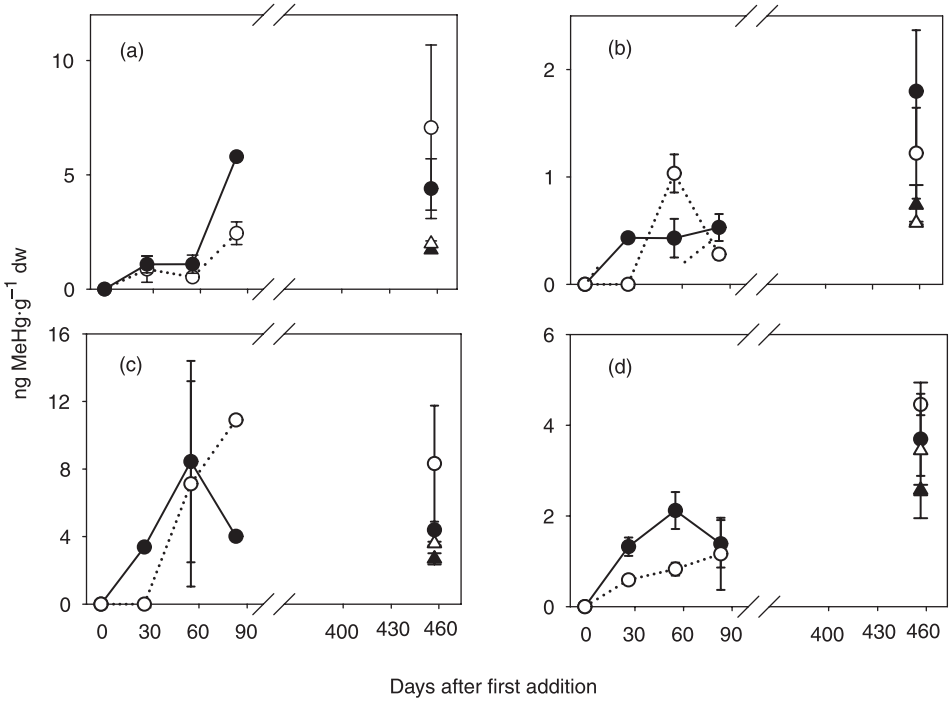


Concentrations of spike MeHg in water were low and often near detection limits. In 2000, spike MeHg was first detected in all enclosures 2–5 weeks after the first addition (Fig. 1*b*). For the 2000 spike, concentrations of MeHg did not diminish in 2001 as did concentrations of THg. There were no obvious differences between enclosures receiving single versus multiple spikes.

In 2000, concentrations of spike THg and MeHg in 0–2 cm sediments increased through time in all enclosures (Figs. 1*c* and 1*d*). Heterogeneity among sediment samples was high, and spike THg and MeHg concentrations were often near detection limits. In 2001, concentrations of 2000 spike THg and MeHg were similar to concentrations measured in September 2000. In September 2001, sediment concentrations of MeHg generated from the 2001 spike were much lower than the September 2000 levels of MeHg produced from the 2000 spike The reason for this difference is uncertain.

In the enclosures, the majority of Hg added as spikes in 2000 and 2001 was lost via evasion to the atmosphere (Amyot et al. 2004; C.C. Gilmour, unpublished data). Mass-balance estimates indicate that by September of each year, an average of 83% ± 3.4% of spike Hg added in 2000 and 85% ± 10.9% of spike Hg added in 2001 was evaded. In September of 2000 and 2001, respectively, an average of 12.2% ± 5.1% and 9.7% ± 9.5% of spike Hg added in the

Fig. 3. Changes in concentrations of the 2000 and 2001 spike methyl mercury (MeHg) in four groups of benthic invertebrates (±1 SEM): (*a*) Chironomidae, (*b*) Pisidiidae (shell removed), (*c*) Hydracarina, and (*d*) Ephemeroptera. Standard errors reflect variability among enclosures receiving either a single Hg isotope addition (E1, E2) or multiple additions (E3, E4). Symbols are as follows: 2000 spike, single addition (solid circles, solid line); 2000 spike, multiple additions (open circles, dotted line); 2001 spike, single addition (solid triangles); 2001 spike, multiple additions (open triangles).



same year remained in the water column, and 4.5% ± 4.5% and 4.5% ± 2.8% was incorporated in enclosure sediments. The remainder (<1%) was accumulated in sediment pore water and in periphyton growing on the enclosure walls. In 2001, <1% of the 2000 spike occurred in the water column and 7.5% ± 3.1% was found in the sediments.

Zooplankton

In 2000, there was considerable variability in concentrations of spike MeHg in zooplankton (Fig. 2). Spike MeHg was first detected within 2–4 weeks of beginning additions, and concentrations increased through the summer. In September 2001 (day 448), the 2000 spike was still readily detectable as MeHg in zooplankton, indicating that the 2000 spike continued to be available to the food web 1 year after its addition to the enclosures.

Benthic invertebrates

In 2000, spike MeHg was detected in all benthic invertebrate groups after 4–8 weeks, and concentrations of spike MeHg generally increased through the summer (Fig. 3). As with the zooplankton, the 2000 spike continued to be available to benthic invertebrates 1 year after addition. In September 2001 (day 462), concentrations of MeHg derived from the 2001 spike were similar to those for the 2000 spike as measured in September 2000 (day 83). The highest concentrations of spike and ambient MeHg were observed in predatory Hydracarina and the lowest concentrations in sphaeriid clams (i.e., Pisidiidae) after shell removal (Fig. 3; Table 3). Chironomids, ceratopogonids, and ephemeropterans (*Hexagenia* spp. and *Caenis* spp.) had intermediate concentrations of spike and ambient MeHg (Table 3).

Fish

Finescale dace showed steady growth in all enclosures in both 2000 and 2001 and approximately doubled in weight by September (2000: initial weight = 2.0 ± 0.3 g, final weight = 3.9 ± 0.5 g; 2001: initial weight = 2.1 ± 0.8 g, final weight = 4.0 ± 1.4 g). The diet of finescale dace was numerically dominated by Cladocera, but the percent contribution by weight was split almost equally between zooplankton and benthic invertebrates (Table 4). Yellow perch had a diet similar to finescale dace, but with an even greater reliance on Cladocera (>60% by weight). Within dates, strong correlations between Hg concentrations and fish weight were not observed.

After only 1 month, newly deposited spike Hg was detected in fish from enclosures that had received both single and multiple spike additions (Fig. 4*a*). Concentrations of the 2000 spike in finescale dace from E1 (single addition) increased rapidly and reached a plateau by July 2000, whereas concentrations of this spike steadily increased through the season in fish from E4 (multiple additions). In September 2001 (day 461), concentrations of the 2001 spike were similar to those observed for the 2000 spike in September 2000 (day 85). In September of 2000, large differences in concentrations of spike Hg in enclosures receiving multiple or single spikes were not evident.

Spike/ambient ratios

Spike/ambient ratios provide an indication of changes in accumulation of spike Hg relative to ambient Hg. We assume that most of the ambient Hg accumulated by biota in the enclosures is derived from past deposition. This is be-

Table 4. The occurrence and percentage by weight of dietary items in stomachs from finescale dace (*Phoxinus neogaeus*) and yellow perch (*Perca flavescens*) captured in E1 and E4 in 2000.

	Finescale dace		Yellow perch	
	Occurrence	% of diet by weight	Occurrence	% of diet by weight
Zooplankton				
Cladocera	3839	47.8	1982	61.9
Copepoda	1	0.4	20	4.1
Benthic invertebrates				
Pisidiidae	9	16.6		
Amphipoda	1	0.2		
Chironomid pupae	12	2.8	57	18.1
Ephemeroptera			1	9.8
Trichoptera	11	6.7	1	3.0
Unidentified Insecta	23	25.1		
Detritus	1	0.4		

Note: Of the 44 finescale dace stomachs examined, six were found to be empty. The stomach contents of seven yellow perch were examined.

cause deposition of ambient Hg to our enclosures was small relative to existing pools in the enclosure sediments and water column and because the enclosures were isolated from ambient Hg inputs from the watershed.

Immediately after additions to single-dose enclosures in both 2000 and 2001, spike/ambient ratios for unfiltered THg in water increased to between 2 and 6 and rapidly diminished thereafter (Fig. 5*a*). In 2000, peak spike/ambient ratios for THg in multiple-dose enclosures varied between approximately 1 and 3 immediately after Hg additions, with lower values at other times. In general, spike/ambient ratios for THg were higher for the 2001 spike because of lower concentrations of ambient THg in that year (Table 3). In 2001, little of the spike Hg added in 2000 remained in the water column, and average spike/ambient ratios were below 0.1 (Table 5). Our detection limit for spike/ambient ratios was ~0.01. Over the course of each summer, average time-weighted spike/ambient ratios for unfiltered spike THg added in that year were between 1.0–3.1, with an overall average of 1.2 (Table 5).

Spike/ambient ratios for MeHg in water were markedly lower than those for THg. Spike/ambient ratios for MeHg in water were always less than 0.3, with overall averages below 0.1 (Fig. 5*b*; Table 5). In 2001, spike/ambient ratios for the 2000 spike did not decrease for MeHg in water as much as they did for THg. This indicates that the 2000 spike continued to be available as MeHg in 2001.

In both 2000 and 2001, spike/ambient ratios for spike THg added in each year and measured in 0–2 cm sediments were far lower than in the overlying water column (overall average = 0.01 ± 0.01; Table 5). This is a result of dilution of spike Hg in the large pool of older ambient Hg in the enclosure sediments. For the 2000 spike, spike/ambient ratios for MeHg in 0–2 cm sediments were similar in magnitude to those for THg. Spike/ambient ratios for sediment MeHg derived from the 2001 spike were lower than those from the 2000 spike. These data suggest that low spike/ambient ratios for MeHg in water reflect the low proportion of spike THg in enclosure sediments.

In 2000, MeHg spike/ambient ratios for zooplankton and benthic invertebrates steadily increased to approximately 0.05 to 0.1 by September (Figs. 5*c*, 5*d*; Table 5). As with MeHg in water, spike/ambient ratios for the 2000 spike in zooplankton and benthic invertebrates did not diminish in 2001. By September 2001, spike MeHg concentrations and spike/ambient ratios for MeHg derived from the 2000 spike increased and were greater for benthic invertebrates than for zooplankton. In part, this may reflect the longer life spans of benthic invertebrates and their longer period of exposure to spike-enriched MeHg. Across all dates and enclosures, average spike/ambient ratios in zooplankton and benthic invertebrates were similar in magnitude to those of MeHg in water for the 2000 and 2001 spikes and in sediments for the 2000 spike (Table 5).

Spike/ambient ratios for finescale dace were also much lower than for dissolved THg (0.04–0.22) (Fig. 4*b*). The highest spike/ambient ratios for finescale dace were observed in fish collected from E1 (single addition) during the first sampling interval in 2000. In subsequent sampling intervals, spike/ambient ratios decreased to average values below 0.1, and in 2000, ratios were similar for fish collected from the multiple and single spike enclosures. In September 2001, spike/ambient ratios for the 2000 spike in finescale dace were similar to those observed 1 year previously and to those observed for zooplankton and benthic invertebrates collected at the same time (Table 5). Once again, this indicates that the 2000 spike continued to be available for uptake by fish 1 year after its addition to the enclosures. Spike/ambient ratios for the 2001 spike in September 2001 were slightly lower than those for the 2000 spike in September 2000, although concentrations of spike Hg in finescale dace were similar.

In September 2001, spike/ambient ratios for the 2000 spike were higher in yellow perch than in finescale dace, possibly

because the yellow perch had lived for 15 months in the enclosures, whereas the finescale dace were added in June 2001.

Discussion

The use of Hg stable isotopes to assess the effects of changes in Hg loading rates

This study was initiated in advance of a whole-lake Hg isotope addition experiment to assess the suitability of using environmentally realistic additions of enriched stable isotopes of Hg to determine the effect of changes in Hg deposition rates on accumulation by fish and other biota. Our results demonstrate that this approach is highly effective. Throughout the experiment, we were easily able to follow the added isotopes as they accumulated in the aquatic food web. The use of stable isotopes greatly enhanced our ability to detect the effect of changes in Hg loading rates. If we had added Hg in a nonisotopic form and sought to determine changes in overall Hg accumulation by biota, we would have been unable to detect any change. This was because of the small relative contribution of added spike Hg to bioaccumulation and background variability in concentrations of ambient Hg.

A further advantage of using Hg stable isotopes was the availability of different isotopes that allowed us to separate the contributions of spikes added in different years. Overall, the dynamics of the Hg spikes added in 2000 and 2001 were very similar. Concentrations and spike/ambient ratios of the two Hg spikes in water, sediments, invertebrates, and fish were similar in September of the year in which they were added. Hence, our results were repeatable among years.

Contribution of newly deposited Hg to Hg accumulation by fish and other biota

Overall, increases in Hg loading to our enclosures contributed to greater MeHg accumulation by fish and other aquatic biota. This suggests that changes in Hg deposition should result in changes in Hg accumulation by fish. Within weeks of beginning additions, the enriched Hg isotopes were detectable as MeHg in water, sediments, zooplankton, and benthic invertebrates. This indicates that a portion of the newly deposited Hg was almost immediately methylated and made available to the food web. Overall, however, increases in MeHg in biota were comparatively small (on average 3%–10%) despite relatively large increases in inorganic Hg loading (200% to >400%). This suggests that most of the Hg accumulated by biota was derived from a large store of Hg deposited in past years.

In our study, we increased inorganic Hg loading to the enclosures considerably over average atmospheric loading at the ELA. Over the course of each summer, average spike/ambient ratios for THg in water were 1.2–1.9 in 2000 and 1.2–6.1 in 2001, which further indicates that the sum of ambient and spike THg increased considerably. Despite these large increases of THg in water, changes in MeHg in biota were much smaller (spike/ambient ratios of 0.04–0.1) and similar for all taxa examined. Spike/ambient ratios for biota were also similar to spike/ambient ratios for MeHg in water and 0–2 cm sediments, suggesting that spike MeHg bioaccumulation was roughly proportional to its availability in the environment. Although we added the equivalent of at least one additional year of Hg loading (and probably more) in each of 2000 and 2001, MeHg accumulation by biota increased by only 3%–10% annually. This suggests that it would take at least 10–30 years for this system to achieve steady state with the new Hg additions, if continued indefinitely. This long response time was driven at least in part because of the small proportion of Hg spike in enclosure sediments, the main site of MeHg production in the enclosures.

Our conclusions are further supported by the observation that spike Hg added in 2000 continued to be as available to biota as MeHg in 2001 as in 2000. Despite large decreases in spike THg in water, there was no sign that the availability of spike MeHg to biota had substantially declined 1 year after its introduction to the enclosures. For organisms with

Fig. 4. (*a*) Changes in concentrations (±1 SEM) of the 2000 and 2001 Hg spike in finescale dace (*Phoxinus neogaeus*). (*b*) Changes in spike ambient ratios (±1 SEM) for the 2000 and 2001 spike in finescale dace. No finescale dace were captured from E2 or E3 in 2000 or from E1 or E2 in 2001. Spike/ambient ratios for finescale dace were corrected for initial ambient body burdens as described in the text. Symbols are as follows: 2000 spike, single addition (E1) (solid circles, solid line); 2000 spike, multiple additions (E3; 2001 only) (open circle); 2000 spike, multiple additions (E4) (open squares, dotted line); 2001 spike, multiple additions (E3) (open triangle); 2001 spike, multiple additions (E4) (open inverted triangle). Standard errors for each enclosure reflect variability among individual fish (not individual enclosures as in previous figures). THg, total mercury (the sum of inorganic and organic Hg).

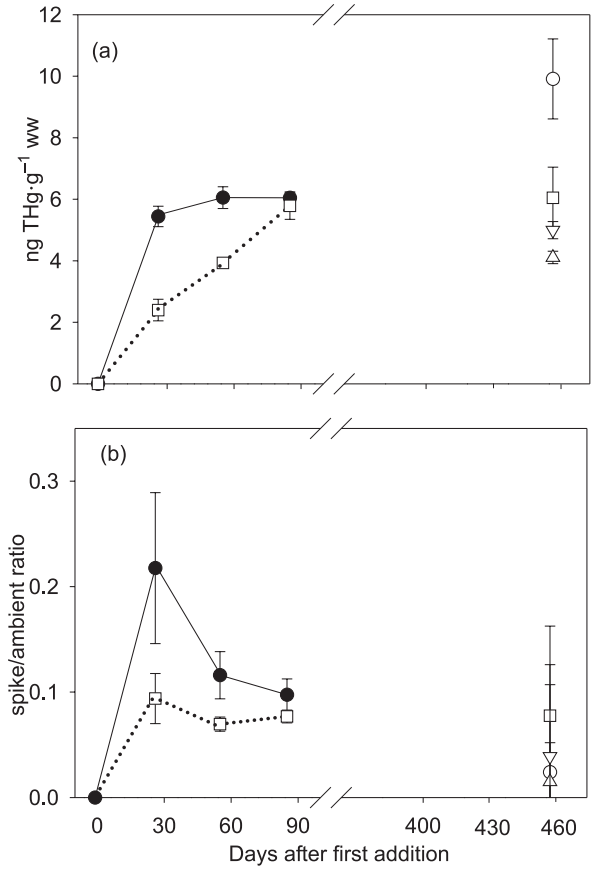


Fig. 5. Changes in spike/ambient ratios (±1 SEM) for (*a*) unfiltered total mercury (THg, the sum of inorganic and organic Hg) in water; (*b*) unfiltered methyl Hg (MeHg) in water; (*c*) MeHg in zooplankton; and (*d*) MeHg in Chironomidae larva. Changes in spike/ambient ratios for other groups of benthic invertebrates were similar to Chironomidae. Standard errors reflect variability among enclosures receiving either a single Hg isotope addition (E1, E2) or multiple additions (E3, E4). Symbols are as follows: 2000 spike, single addition (solid circles, solid line); 2000 spike, multiple additions (open circles, dotted line); 2001 spike, single addition (solid triangles, solid line); 2001 spike, multiple additions (open triangles, dotted line).

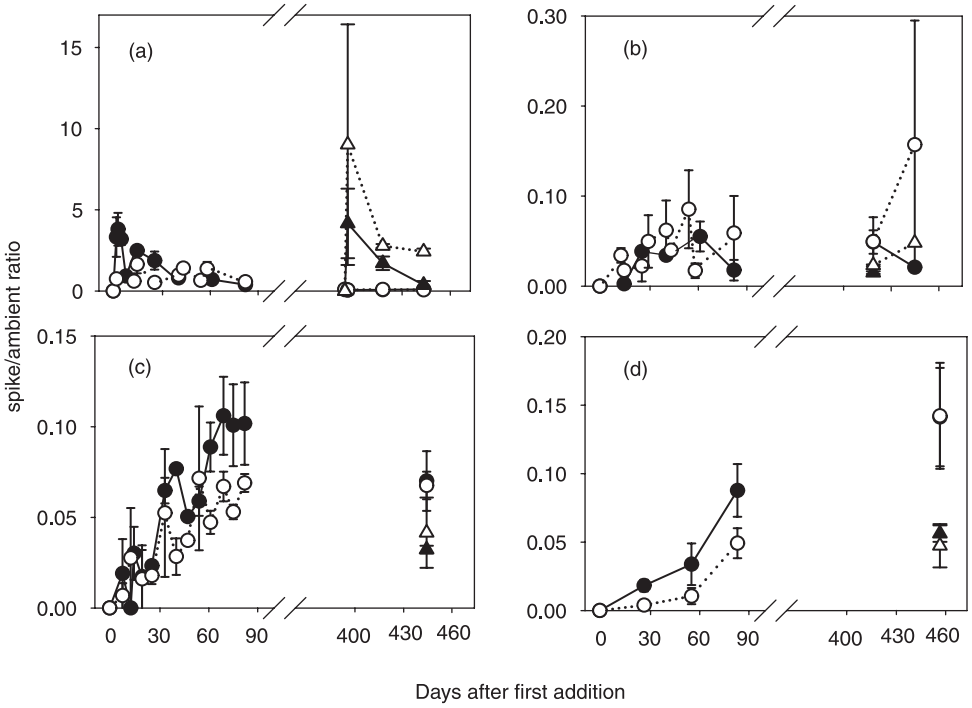


Table 5. Average spike/ambient ratios in the study enclosures in 2000 and 2001.

	Year 2000	Year 2001	
	2000 spike	2000 spike	2001 spike
Water			
Unfiltered THg	1.16 (0.18)	0.08 (0.01)	3.14 (1.06)
Unfiltered MeHg	0.03 (0.01)	0.09 (0.03)	0.03 (0.01)
Sediments (0–2 cm)			
THg	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)
MeHg	0.01 (0.01)	0.02 (0.01)	0.002 (0.001)
Invertebrate MeHg			
Zooplankton	0.08 (0.02)	0.07 (0.01)	0.04 (0.01)
Ceratopogonidae	0.05 (0.01)	—	—
Chironomidae	0.07 (0.02)	0.14 (0.02)	0.05 (0.01)
Ephemeroptera	0.05 (0.01)	0.09 (0.01)	0.07 (0.01)
Hydracarina	0.06 (0.02)	0.12 (0.02)	0.06 (0.01)
Pisidiidae	0.04 (0.01)	0.11 (0.02)	0.05 (0.01)
Fish THg			
Finescale dace (*Phoxinus neogaeus*)	0.09 (0.01)	0.05 (0.02)	0.03 (0.01)
Yellow perch (*Perca flavescens*)	—	0.13 (0.03)	—

Note: Data for Hg concentrations in water are time-weighted June–September averages, whereas all other data are for September samples only. Ceratopogonidae were not encountered in the 2001 samples. Spike/ambient ratios for finescale dace were corrected for initial ambient muscle burdens as discussed in the text. Numbers in brackets are 1 standard error of the mean and reflect variation among enclosures. THg, total Hg (the sum of inorganic and organic Hg); MeHg, methyl Hg.

longer life spans such as benthic invertebrates and yellow perch, spike MeHg concentrations increased from 2000 to 2001. For organisms with short life spans (e.g., zooplankton) or for organisms introduced to the enclosures each year (finescale dace), spike Hg concentrations and spike/ambient ratios were similar in September 2000 and 2001. Given the comparatively short duration of our experiment, extrapolations beyond 1 year are not possible.

Many features of natural lakes differ substantially from our enclosures. As a result, natural lakes may respond differently to changes in Hg deposition. For example, the enclosures were shallow and lacked anoxic water, which may support substantial Hg methylation (Eckley et al. 2005). We also observed considerable photoreduction and evasion of Hg from the enclosures, which decreased the amount of Hg available for methylation (Amyot et al. 2004). It is uncertain whether the evasion rates observed in our enclosures were anomalously high as compared with natural lakes. While these factors may result in faster responses in natural lakes, others may result in a slower response relative to our enclosures. For example, a considerable proportion of Hg in lakes is derived from the surrounding watershed, as opposed to direct deposition to the lake surface, as in our study. There are many potential delays in the transfer of newly deposited Hg from a catchment to the lake itself (e.g., Hintelmann et al. 2002). Average temperatures were higher in our enclosures than in many lakes, which may increase relative rates of methylation. In addition, large, old fish in natural lakes may take many years to depurate MeHg accumulated during previous growth. Notwithstanding these potentially important differences between enclosures and natural lakes, our data may be applicable to shallow systems dominated by littoral sediments. Clearly, further study is required.

Single versus multiple additions

By September of both 2000 and 2001, there were no large differences in spike Hg concentrations in biota from enclosures receiving single or multiple additions. Overall, this suggests that long-term results (>6 months) were not strongly affected by the method of spike addition (multiple versus single spikes). Presumably this is because spike Hg was diluted into a comparatively large pool of ambient Hg and because there were time lags in the conversion of inorganic Hg to MeHg and subsequent accumulation by biota.

In contrast with the September results, Hg concentrations in finescale dace collected during the first 8 weeks of 2000 had higher concentrations of spike Hg in the single dose enclosure (E1) than in the multiple dose enclosure (E4). This result is surprising because there was no indication of higher concentrations of spike MeHg in invertebrates collected from E1 versus E4 at the time. In addition, spike/ambient ratios for finescale dace in the single dose enclosure were much higher than for their food resources: benthic invertebrates and zooplankton. One possible explanation for this result is that Hg in fish was measured as THg, whereas Hg in invertebrates was measured as MeHg. Under natural conditions, >90% of the Hg in fish is MeHg and THg is a sufficient measure of MeHg concentrations (Bloom 1992). In the early stages of our experiment, however, concentrations of inorganic spike Hg were briefly increased to very high levels relative to MeHg because there was insufficient time for methylation of the spike to achieve steady state with the new loading rates. As a result, much of the spike Hg incorporated by finescale dace in the first few weeks may have been inorganic Hg.

Although our use of THg may have biased our short-term results for fish, we believe that by September of each year, these biases were greatly reduced. Within weeks of addition, much of the added inorganic Hg was lost from the enclosures and probably newly accumulated inorganic Hg was largely depurated by fish. The model of Trudel and Rasmussen (1997) indicates that inorganic Hg is depurated by a 3 g fish with a half-life of approximately 10 days. In contrast, MeHg depuration would have a half-life of >250 days in similarly sized fish. With time, an increasingly larger proportion of the spike Hg in the enclosures was also methylated and relative incorporation of spike MeHg by fish presumably became increasingly important. Turner and Rudd (1983) and Hecky et al. (1991) found that >60% and >85%, respectively, of newly accumulated Hg added as radioactive ^{203}Hg occurred as MeHg in fish collected from large enclosures similar to ours after 60 days. By September in our enclosures, spike/ambient ratios for fish and invertebrates were very similar. Concentrations and spike/ambient ratios for the 2000 spike were also similar in September 2000 and 2001, further suggesting that the system was moving toward a new steady state with respect to MeHg–THg dynamics.

In summary, our study clearly demonstrates the utility of using stable isotopes of Hg to examine Hg accumulation in aquatic communities. The enriched isotopes were clearly detectable in biota, and the availability of different isotopes facilitated among-year comparisons. Our data suggest that changes in deposition of inorganic Hg will result in changes in MeHg accumulation by fish and other biota. Although long-term changes in Hg deposition should result in changes in MeHg accumulation by biota in such systems, it may take much more than a decade for steady-state conditions to be achieved. More study is required to determine to what extent these data from enclosures can be extrapolated to natural lakes.

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References

Amyot, M., Southworth, G., Lindberg, S.E., Hintelmann, H., Lalonde, J.D., Ogrinc, N., Poulain, A.J., and Sandilands, K.A. 2004. Formation and evasion of dissolved gaseous mercury in large enclosures amended with $^{200}HgCl_2$. Atmos. Environ. **38**: 4279–4289.

Bloom, N.S. 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapor atomic fluorescence detection. Can. J. Fish. Aquat. Sci. **46**: 1131–1140.

Bloom, N.S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquat. Sci. **49**: 1010–1017.

Bodaly, R.A., and Fudge, R.J.P. 1999. Uptake of mercury by fish in an experimental boreal reservoir. Arch. Environ. Contam. Toxicol. **37**: 103–109.

Downs, S.G., Macleod, C.L., and Nester, J.N. 1998. Mercury in precipitation and its relations to bioaccumulation in fish: a literature review. Water Air Soil Pollut. **108**: 149–187.

Eckley, C.S., Watras, C.J., Hintelmann, H., Morrison, K., Kent, A.D., and Regnell, O. 2005. Mercury methylation in the hypolimnetic waters of lakes with and without connection to wetlands in northern Wisconsin. Can. J. Fish. Aquat. Sci. **62**: 400–411.

Eddy, J.B. 2000. Estimation of the abundance, biomass and growth of a northwestern Ontario population of finescale dace (*Phoxinus neogaeus*), with comments on the sustainability of local commercial baitfish harvests. Master of Natural Resource Management thesis, University of Manitoba, Winnipeg, Man.

Fitzgerald, W.F., Engstrom, D.R., Mason, R.P., and Nater, E.A. 1998. The case for atmospheric mercury contamination in remote areas. Environ. Sci. Technol. **32**: 1–7.

Gilmour, C.C., Riedel, G.S., Ederington, M.C., Bell, J.T., Benoit, J.M., Gill, G.A., and Stordal, M.C. 1998. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. Biogeochemistry, **40**: 327–345.

Hecky, R.E., Ramsey, D.J., Bodaly, R.A., and Strange, N.E. 1991. Increased methylmercury contamination in fish in newly formed freshwater reservoirs. *In* Advances in mercury toxicology. *Edited by* T. Suzuki, N. Imura, and T.W. Clarkson. Plenum Press, New York. pp. 33–52.

Hintelmann, H., and Ogrinc, N. 2003. Determination of stable mercury isotopes by ICP/MS and their application in environmental studies. *In* Biogeochemistry of environmentally important trace elements. *Edited by* Y. Cai and C.O. Braids. American Chemical Society, Washington, D.C. pp. 321–338.

Hintelmann, H.H., Harris, R., Heyes, A., Hurley, J.P., Kelly, C.A., Krabbenhoft, D.P., Lindberg, S., Rudd, J.W.M., Scott, K.J., and St. Louis, V.L. 2002. Reactivity and mobility of new and old mercury deposition in a boreal forest ecosystem during the first year of the METAALICUS study. Environ. Sci. Technol. **36**: 5034–5040.

Horowitz, A.J., Demas, C.R., Fitzgerald, K.K., Miller, T.L., and Rickert, D.A. 1994. U.S. Geological Survey protocol for collection and processing of surface-water samples for the subsequent determination of inorganic constituents in filtered water. US Government Printing Office, Washington, D.C. USGS Open File Rep. 94-539.

Horvat, M., Liang, L., and Bloom, N.S. 1993. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples. Anal. Chim. Acta, **282**: 153–168.

Hrabik, T.R., and Watras, C.J. 2002. Recent declines in mercury concentration in a freshwater fishery: isolating the effects of deacidification and decreased atmospheric mercury deposition in Little Rock Lake. Sci. Tot. Environ. **297**: 229–237.

Jackson, T.A. 1997. Long-range atmospheric transport of mercury to ecosystems, and the importance of anthropogenic emissions — a critical review and evaluation of the published evidence. Environ. Rev. **5**: 99–120.

Johansson, K., Aastrup, M., Andersson, A., Bringmark, L., and Iverfeldt, A. 1991. Mercury in Swedish forest soils and waters — assessment of critical load. Water Air Soil Pollut. **56**: 267–281.

Mason, R.P., Fitzgerald, W.F., and Morel, F.M. 1994. The biogeochemical cycling of elemental mercury: anthropogenic influences. Geochim. Cosmochim. Acta, **58**: 3191–3198.

Morrison, K.A., and Watras, C.J. 1999. Mercury and methyl mercury in freshwater seston: direct determination at picogram per litre levels by dual filtration. Can. J. Fish. Aquat. Sci. **56**: 760–766.

North American Implementation Task Force on Mercury. 2000. North American regional action plan on mercury: phase II [online]. Available from http://www.cec.org/programs_projects/pollutants_health/smoc/pdfs/Hgnarap.pdf [accessed November 2005].

Orihel, D.M. 2005. The effects of changes in atmospheric mercury deposition on the bioaccumulation of mercury in fish. Master of Natural Resource Management thesis, University of Manitoba, Winnipeg, Man.

Paterson, M.J., Rudd, J.W.M., and St. Louis, V. 1998. Increases in total and methylmercury in zooplankton following flooding of a peatland reservoir. Environ. Sci. Technol. **32**: 3868–3874.

Pickhardt, P.C., Folt, C.L., Chen, C.Y., Klaue, B., and Blum, J.D. 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. Proc. Natl. Acad. Sci. U.S.A. **99**: 4419–4423.

Pickhardt, P.C., Folt, C.L., Chen, C.Y., Klaue, B., and Blum, J.D. 2005. Impacts of zooplankton composition and algal enrichment on the accumulation of mercury in an experimental freshwater food web. Sci. Tot. Environ. **339**: 89–101.

Stainton, M.P., Capel, M.J., and Armstrong, F.A.J. 1977. The chemical analysis of fresh water. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. No. 25.

St. Louis, V.L., Rudd, J.W.M., Kelly, C.A., Bodaly, R.A., Paterson, M.J., Beaty, K.G., Hesslein, R.H., Heyes, A., and Majewski, A.R. 2004. The rise and fall of mercury methylation in an experimental reservoir. Environ. Sci. Technol. **38**: 1348–1358.

Trudel, M., and Rasmussen, J.B. 1997. Modeling the elimination of mercury by fish. Environ. Sci. Technol. **31**: 1716–1722.

Turner, M.A., and Rudd, J.W.M. 1983. The English–Wabigoon River system. III. Selenium in lake enclosures: its geochemistry, bioaccumulation, and ability to reduce mercury bioaccumulation. Can. J. Fish. Aquat. Sci. **40**: 2228–2240.

Wiener, J.G., Krabbenhoft, D.P., Heinz, G.H., and Scheuhammer, A.M. 2003. Ecotoxicology of mercury. *In* Handbook of ecotoxicology. Lewis Publishers, Boca Raton, Fla. pp. 409–463.