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UPTAKE OF METHYL MERCURY BY THE FLOATER MUSSEL, *PYGANODON GRANDIS* (BIVALVIA, UNIONIDAE), CAGED IN A FLOODED WETLAND

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Abstract—A 16.7-ha wetland with a pond at the Experimental Lakes Area, northwestern Ontario, was experimentally flooded to determine causes of elevated waterborne methyl mercury (MeHg) associated with impoundment. Unionid mussels, *Pyganodon grandis* (formerly *Anodonata grandis grandis*) were caged in the experimental pond before and after flooding to determine their ability to monitor the elevated waterborne MeHg. Mussels were also caged in a reference wetland pond and in the lake from which they were collected (source lake). Background levels of MeHg in source lake mussel body parts were in the order mantle < gill or visceral remains < foot or kidney and ranged from 108 to 618 ng/g dry weight. Caging in the source lake did not alter MeHg concentration in any body part. Mussels transplanted to the ponds for 90 d showed statistically significant increases above background MeHg in the mantle and visceral remains in the preflood experimental pond (waterborne MeHg 0.09 ng/L) and in the mantle, visceral remains, foot, and kidney in the reference pond (waterborne MeHg 0.24 ng/L). The visceral remains of mussels in the reference pond contained higher levels of MeHg than did those in the preflood experimental pond. Flooding increased waterborne MeHg from 0.1 to 2.3 ng/L and resulted in an increase in MeHg and total Hg (THg) in the mantle, foot, and visceral remains of mussels in the experimental pond. Inexplicably, mussels caged in a hypoxic environment in the experimental pond lost MeHg and THg from all body parts. We concluded that not only can *P. grandis* monitor elevation in waterborne MeHg with flooding, but the MeHg levels in mussels also reflect small differences in background levels of natural MeHg in ponds.

Keywords—Methyl mercury Total mercury Unionid mussels Experimental Lakes Area Flooding

INTRODUCTION

The role of freshwater mussels, particularly unionids, as biomonitors for heavy metal and organic environmental contamination has been explored over several decades [1–6]. There are a number of features that make unionids suitable for this purpose. These include a wide geographical range, high species diversity (decreasing towards northern latitudes), long life, and a large range of body size. Unionids are usually littoral in habitat, relatively easy to collect, and relatively sedentary. They accumulate contaminants and are tolerant of high body burdens of many contaminants [7,8]. They are amenable to caging and thus can be deployed for known time periods in environments in which they do not naturally occur.

The accumulation of heavy metals by unionids is well documented [7–10]. Nevertheless, most of the studies have either been laboratory-based using supra-environmental concentrations or based on observing mussels from heavily contaminated field sites. Accumulation studies on mussels at the Experimental Lakes Area (ELA), northwestern Ontario, are distinguished from most other studies by the low levels of heavy metals to which the mussels are exposed in ecologically realistic whole lake experiments [11].

In this paper, we examine the potential for mussels to serve as a biomonitor of elevated waterborne methyl mercury (MeHg) associated with flooding. In an experiment, referred to as the Experimental Lakes Area Reservoir Project (ELARP) [12], a wetland is being flooded for better understanding of the following problems associated with man-made reservoirs: (1) the evolution of greenhouse gases, and (2) the causes of elevated MeHg concentrations in fish taken from reservoirs [13,14]. The objectives of this study were, first, to measure background MeHg and total Hg (THg) concentrations in five body parts of mussels. Second, we examined the effects of caging of mussels on the Hg concentrations by caging them in the lake from which they were collected. Third, we transplanted mussels in cages to the experimental wetland pond before flooding, as well as to a nearby reference wetland pond to observe the effects of wetland environments on Hg concentrations. Fourth, we transplanted mussels in cages to the experimental wetland after flooding to observe the effect of elevated environmental MeHg on the Hg concentrations of mussels. The impacts of caging on mussel growth, weight, fecundity, and survival are also noted.

MATERIALS AND METHODS

Experimental sites

Lake 979 is a riverine wetland with a central pond (2.39-ha area) at 49°38′N latitude and 93°43′W longitude in the Experimental Lakes Area, northwestern Ontario, a research preserve described by Brunskill and Schindler [15]. The wetland area, including the pond, is 16.7 ha and the watershed area is 98.1 ha. Prior to flooding, in 1991 to 1993, the pond water had MeHg at 0.09 ± 0.04 ng/L (mean ± SD) and THg at 2.6 ± 1.5 ng/L [16]. Lake 979 receives water from upstream Lake 240, the outflow of which contains MeHg at 0.06 ± 0.03 ng/L and THg at 1.5 ± 0.81 (n = 58 for both determinations) [16].

Lake 632, a headwater wetland (4.25-ha area) including a pond (0.86-ha area) having a watershed area of 42 ha located at 49°41′N and 93°48′W, served as a reference system. In 1991 to 1993, the pond averaged MeHg at 0.24 ± 0.07 ng/L and THg at 3.3 ± 1.2 ng/L [16]. Other water chemistry parameters of both ponds are given in Table 1.

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Experimental Lakes Area Reservoir Project Contribution 13.

Table 1. Morphometry and representative chemical data from the lakes in this study

Parameter	Lake 104[a]	Lake 632[b]	Lake 979[c]
Surface area (ha)	8.1	0.86	2.39[d]
Maximum depth (m)	4.8	1.3	1.45
Conductivity (μS/cm at 25°C)	27	18	31
pH	7.04	5.99	6.83
Alkalinity (μmoles/L)	154	47	126
Chlorophyll a (μg/L)	2.86	n/d[e]	n/d
Suspended carbon (μg/L)	460	1,030	710
Dissolved inorganic C (μmoles/L)	170	60	160
Dissolved organic C (μg/L)	820	1,120	810
Suspended N (μg/L)	40	75	67
Total dissolved N (μg/L)	n/d	370	410
NO_2N (μg/L)	n/d	2	1
NH_4N (μg/L)	n/d	11	17
Suspended P (μg/L)	2	5	6
Total dissolved P (μg/L)	3	3	5
Ca^{2+} (mg/L)	2.95	1.67	2.56
Na^{+} (mg/L)	1.06	1.06	1.33
K^{+} (mg/L)	0.53	0.21	0.61
Mg^{2+} (mg/L)	0.80	0.53	1.00
Fe (mg/L)	0.09	0.04	0.09
Mn (mg/L)	0.01	n/d	0.01
Soluble reactive silicon (mg/L)	1.010	0.660	0.533
Cl^{-} (mg/L)	0.29	0.11	0.47
SO_4^{2-} (mg/L)	2.11	2.59	4.20

[a]Water sampled on June 24, 1991.
[b]Water sampled on August 8, 1991. Data supplied by the ELARP project.
[c]Water sampled on August 15, 1991. Data supplied by the ELARP project.
[d]Prior to flooding.
[e]n/d = no data.

Both systems were studied in their natural condition in 1992. Beginning on June 23, 1993, Lake 979 was flooded, whereas Lake 632 remained unmanipulated. Approximately 1 month after the flooding, MeHg increased to about 1.9 ng/L. During the July to October 1993 period, after flooding, when the mussels were caged at the center of Lake 979, MeHg in the water varied from 0.5 to 2.3 ng/L [12], and averaged 1.38 ± 0.51 ng/L [16]. Total Hg averaged 4.5 ± 1.7 ng/L [16]. Lake 979 is referred to below as the experimental pond and Lake 632 as the reference pond.

Collection and caging of mussels

Unionids do not occur in these wetland ponds. Therefore, the floater mussel, *Pyganodon grandis* (formerly *Anodonta grandis grandis* (Say)) was collected from Lake 104 (49°41′N, 93°50′W), where they are abundant. Water chemistry of Lake 104 is also given in Table 1. Methyl Hg and THg were not measured in Lake 104. Mussels were collected by snorkel divers from about 1.5 to 2-m water depth in the same area of the lake in 1992 and 1993. Lake 104 is referred to below as the source lake.

Mussels were introduced into the experimental and reference ponds in cages suspended so that the mussels were at 1-m water depth, unless stated otherwise. Cages were made of an unfinished wood frame, 36 cm on each side, fastened together with wooden dowels. Plastic mesh, with openings of 4 mm × 4 mm, was sewn to the frame with nylon fishing line.

The experimental design is given in Table 2. In July 1992, 52 mussels were collected from the source lake, 12 of which were immediately dissected (termed fresh mussels) for measurement of background MeHg and THg. Two cages containing 10 mussels each were placed near the centers of the reference and experimental ponds (40 mussels). One cage from each pond was retrieved in August and the other in October.

In July 1993, 52 mussels were collected from the source lake. Ten were dissected fresh and eight mussels were placed in each of two cages in each of the reference and experimental ponds. Both cages in the reference pond were suspended near the center, but in the experimental pond one cage was suspended at the center and one in an area over flooded peatland. The bottom of the latter cage was at about 0.5-m depth. This cage was retrieved early because two of the eight mussels had died. The other three cages in the reference and experimental ponds were sampled in October.

To determine the changes in MeHg and THg contents of mussels due to season, mussels were collected and dissected fresh from the source lake in October 1993. To determine effects

Table 2. Experimental design

Dates of collection or caging	No. of mussels	ID	Duration (d)	Purpose
Source lake				
July 10, 1992	12	Fresh	n/a[a]	Precaging background MeHg concentrations, 1992
July 14, 1993	10	Fresh	n/a	Precaging background MeHg concentrations, 1993
Oct. 12, 1993	10	Fresh	n/a	Effect of season on background MeHg concentrations
July 16–Oct. 12, 1993	10	Cage	88	Effect of caging on MeHg concentrations
Reference pond				
July 10–Oct. 8, 1992	10	Cage A	90	Effect of caging in unmanipulated wetland, long, 13 weeks
July 10–Aug. 27, 1992	10	Cage B	48	Effect of caging in unmanipulated wetland, short, 7 weeks
July 14–Oct. 12, 1993	8	Cage A	90	Effect of caging in unmanipulated wetland, year-to-year variability
July 14–Oct. 12, 1993	8	Cage B	90	Replicate of Cage A
Experimental pond				
July 10–Oct. 8, 1992	10	Cage A	90	Effect of caging in experimental wetland prior to flooding, 13 weeks
July 10–Aug. 27, 1992	10	Cage B	48	Effect of caging in experimental wetland prior to flooding, 7 weeks
July 14–Oct. 11, 1993	8	Cage A	89	Effect of flooding on MeHg concentrations, center of pond
July 14–Aug 23, 1993[b]	8	Cage B	40	Effect of flooding on MeHg concentrations, over flooded peatland

[a]n/a = not applicable.
[b]Planned duration 90 d, but removed early because of mortality.

of caging on Hg contents, 10 mussels were caged in the source lake from July to October 1993 at the same depth as in the ponds.

Processing of mussels

After collection, mussels were held in the laboratory in moist conditions (without standing water) for 4 to 30 h at a temperature close to that of the habitat. Prior to placement in cages or to dissection, mussels were weighed live and the shell was measured along three axes using vernier calipers as previously described [17]. For live weighing, mussels were placed in ELA tap water for 0.5 to 3 h to allow them to ventilate. Actively ventilating mussels were removed from water, blotted dry with paper towels, and weighed immediately to the nearest 0.01 g. This ensured that the mantle cavity was full of water, a requirement for reproducible live weights.

At the time of dissection, the presence of larvae (glochidia) was noted in the outer gill lamella (marsupium). Glochidia appear in the gill in July and are incubated until the following early spring. Mussels were dissected using stainless steel utensils and a scalpel with a surgical blade into mantle; gills, including labial palps; foot; kidney; and the rest of the body, here termed visceral remains, including body wall, gut with crystalline style, hepatopancreas, and gonad. Body parts were freeze-dried on a Lab Con Co. Freeze Dry 5 (Fisher Scientific Co., Winnipeg, MB, Canada) at −68 to −75°C and a pressure of 0.5 to 1 Pa for at least 120 h. In some cases, kidney was pooled from two or three mussels in the same treatment to provide sufficient sample mass for analysis.

When collected from Lake 104, the 114 mussels in this study ranged in length from 76.9 to 122.0 mm and in weight from 37.9 to 125.8 g. Means ± SE of the 6 to 12 mussels in each of the 12 groups of fresh or caged mussels ranged from 101.1 ± 2.7 to 105.9 ± 2.8 mm in length and from 80.0 ± 3.9 to 91.2 ± 8.4 g in live weight. There were no differences among the 12 groups in mean length, height, thickness (obesity), or live weight by one-way ANOVA ($p > 0.05$ for each dimension and weight) using SYSTAT [18].

Determination of methyl mercury

Protein-bound organic Hg was determined in the body parts of mussels by the Freshwater Institute Metals Analytical Laboratory using a method developed by the Department of Fisheries and Oceans Inspection Branch, Central and Arctic Region, Freshwater Institute. The organic Hg was released by homogenizing a sample of freeze-dried ground tissue in a solution of acidic NaBr (30% w/v) and $CuSO_4$ (2.5% w/v). A 3:2 mixture of methylene chloride and hexane was used to partition the resulting organic mercury bromide. An aliquot was digested overnight at 180°C in a 4:1 mixture of H_2SO_4 and HNO_3, and oxidized Hg was converted to the elemental state with a $(NH_2OH)_2 \cdot H_2SO_4$–$SnCl_2$–NaCl reducing solution. The Hg^0 was partitioned into air and determined by flameless atomic absorption at 253.7 nm.

Appropriate standards and reference material were analyzed with each group of samples. Reference material included National Research Council of Canada (NRCC) dogfish muscle (Dorm-1) and National Bureau of Standards (U.S. Department of Commerce, Gaithersburg, MD, USA) oyster tissue (Standard Reference Material 1566a). Certified ranges for MeHg for Dorm-1 were 671 to 793 ng/g and for oyster tissue were 42 to 72 ng/g. Methyl Hg determinations on reference material included in sample runs were normally within the certified ranges. Concentrations of MeHg are expressed as ng Hg/g dry weight of tissue.

The organic Hg determined in mussel body parts in this study is termed MeHg. It is assumed that other organic Hg forms, if present, are negligible. Analyzed by GC cold vapor atomic fluorescence spectroscopy, the organic Hg in ELA water samples has been determined to be predominately MeHg (J.W.M. Rudd, personal communication).

Total Hg in body parts was determined by the method of Henzel and Jamieson [19] modified from Armstrong and Uthe [20]. Total Hg is expressed as ng Hg/g dry weight of tissue.

The ratio MeHg : THg is expressed here as percent. Concentrations of MeHg and THg, and their ratio, in whole body were calculated by compositing values from the individual body parts as follows

$$\text{whole body MeHg (or THg) concn.} = \frac{\Sigma\,[C_i \cdot W_i]}{\Sigma\, W_i}$$

where C_i is the concentration of MeHg or THg in ng/g dry weight in the ith body part, and W_i is the dry weight of the ith body part. The percent contribution of the MeHg in a body part to the total MeHg burden was calculated as

$$\text{MeHg as \% of total MeHg burden} = \frac{C_i \cdot W_i}{\Sigma\,[C_i \cdot W_i]} \cdot 100$$

Variability in time for gut evacuation between collection and dissection did not affect the MeHg concentration of the visceral mass. Mussels from replicate cages A and B, taken from the reference pond in October 1993, were dissected starting 5 and 30 h, respectively, after removal from the pond. Mean MeHg concentration in the visceral remains of the 30-h evacuated mussels was not significantly lower than those of the 5-h evacuated mussels (t test for equal variances, $t = 1.06$; $p = 0.156$).

Statistics

Standard statistics were calculated on dimensions, live weight, MeHg and THg concentrations, and their ratio MeHg : THg in the body parts and composite whole body. Because all the mussels originated from the same population, mean dimensions and live weights were compared among the groups of fresh and ready-to-be caged mussels by a one-way ANOVA [18]. Although mussels were not marked, measurements on individuals after caging were matched with the measurements before caging. Change in dimensions and live weights of individual mussels before and after caging was tested, within each group of caged mussels, with a paired two-tailed t test for means (Quattro-Pro Version 2 for Windows). The relationship between MeHg concentration in each of the five body parts and the composite body and body size was determined by regressing MeHg concentrations against live weight. Statistical significance was judged by r^2 associated with $p < 0.05$. For almost all cases, concentrations of MeHg bore no relationship with live weight. Therefore, in this study the data on Hg levels were not corrected for body size. Although it is common to find a relationship between whole body MeHg and body size, the size range of mussels in this study may have been too narrow to determine such a relationship.

For each body part, seasonal differences and differences due to caging in MeHg, THg, and MeHg : THg of mussels from the source lake and the reference and experimental ponds prior to flooding were determined using SAS version 6.08 ANOVA and the Least Square Means test for differences [21]. The statistical significance of the changes in MeHg, THg concentrations, and

Table 3. Changes in sizes of mussels with caging and percent with glochidia. Values are means ± SE. *p* is the probability that the changes in dimensions are equal to 0

	Length (mm)			Weight (g)			% With glochidia
Dates	After caging	Mean % change	*p*	After caging	Mean % change	*p*	After caging
Source lake							
July–Oct. 1993	105.2 ± 2.7	+1.13	<0.0001	90.2 ± 6.1	+0.10	0.937	20
Reference pond							
July–Aug. 1992	105.7 ± 3.2	+0.50	0.007	87.5 ± 5.4	−1.39	0.187	25
July–Oct. 1992	106.4 ± 2.8	+0.43	0.006	85.9 ± 4.9	−3.32	0.005	20
July–Oct. 1993, cage A	104.1 ± 3.4	+0.98	0.002	79.3 ± 6.7	−3.98	0.001	25
July–Oct. 1993, cage B	104.7 ± 2.3	+0.60	0.052	87.0 ± 5.0	−1.62	0.047	25
Experimental pond							
July–Aug. 1992	105.8 ± 3.0	+0.69	0.005	86.6 ± 5.7	−1.51	0.062	30
July–Oct. 1992	105.1 ± 1.9	+0.49	0.0007	83.9 ± 3.8	−4.85	0.048	20
July–Oct. 1993	103.2 ± 3.5	+0.79	0.018	82.6 ± 7.9	−0.85	0.654	38
July–Aug. 1993	105.6 ± 3.1	−0.10	0.744	91.6 ± 8.6	+0.49	0.060	33

MeHg : THg with flooding was determined by comparing these parameters in mussels from the two ponds in 1992 and 1993 in a two-way ANOVA using site and year as factors and calculating the interaction between site and year [21]. When required, data were log-transformed and tested for normality using the Shapiro–Wilk statistic, a ratio between the best estimator of the variance and the corrected sum of squares estimator of the variance. Significance was accepted at the $p = 0.05$ level.

RESULTS

Effects of caging on mussels

Ten mussels caged in the source lake for 88 d in 1993 all survived and increased a small amount in shell length but did not change in weight (Table 3). All four groups of mussels caged in the reference pond in 1992 and 1993 grew significantly in length (Table 3; $p = 0.052$ in one case). All four groups lost small amounts of weight; losses were statistically significant for three groups (Table 3).

Three of the four groups of caged mussels in the experimental pond in 1992 and 1993 grew significantly in length (Table 3). Three of the four groups lost weight, although the change was statistically significant for only one. Mussels in the cage over flooded peatland in 1993 were retrieved on August 23, rather than in October as planned, because two of the eight mussels had died. Dissolved O_2 at this site was effectively zero at the peat–water interface (J.W.M. Rudd, unpublished data). The mussels were within 0.25 m of this interface and undoubtedly were exposed to very low O_2. The six surviving mussels (July–Aug. 1993 in Table 3) showed no significant change in shell length or weight.

Mussels collected and caged in mid-July already bore early glochidia. Upon dissection in August and October, mussels had approximately the same proportion of fecund individuals, whether they were caged or fresh. Of the groups freshly collected from the source lake in July 1992 and July and October 1993, 20 to 50% bore glochidia. Of the groups dissected in October 1992 or 1993 after caging in the source lake or the wetland ponds, 20 to 38% bore glochidia (Table 3). Thus, caging did not appear to result in unseasonal expulsion of glochidia.

Concentrations of MeHg and THg in source lake mussels

In mussels freshly collected from the source lake, background MeHg concentrations tended to be in increasing order: mantle < gill or visceral remains < foot, kidney, except in October 1993 when gill was lowest (Table 4). Low MeHg concentrations were associated with gills bearing glochidia (data not shown). In mantle, gill, and visceral remains, MeHg comprised about 40 to 60% of THg. In kidney, MeHg was 15 to 36% of THg, and in the foot, MeHg was as high as 93% of THg (Table 4). Concentrations of MeHg and THg and their ratio in the visceral remains were similar to those in the composite whole body, making this body part a useful surrogate for the whole body mass (Table 4).

In 1993, there was a seasonal effect in the Hg concentrations in some body parts. Comparing source lake mussels in October 1993 with those in July 1993 (Table 4), MeHg and THg increased significantly in the mantle (*t* test, $p = 0.006$ and 0.007, respectively). Methyl Hg, but not THg, declined in the gill ($p = 0.0001$) and the kidney ($p = 0.046$). Seasonal differences in the foot and visceral remains were not significant.

Mussels caged in the source lake from July to October 1993 (data not shown) showed no significant changes in MeHg or THg in any body part or composite body compared with mussels freshly collected from the source lake in October 1993 (ANOVA, $p > 0.05$) Therefore, the process of caging did not alter MeHg or THg concentrations.

Changes in MeHg and THg in mussels transplanted from a lake to wetland ponds

The transplantation of mussels in 1992 from the source lake to the reference and experimental ponds resulted in elevation of MeHg and THg in mantle, foot, kidney (MeHg only), and visceral remains (Table 5). Mantle showed the most consistent increases. Part of the increase may be due to seasonal change. There were no significant changes in gill (data not shown). Increases in body parts were more marked in October 1992 than in August (Table 5) and more of the increases were statistically significant in the reference pond than in the preflooded experimental pond. As a result, the MeHg and THg concentrations in the visceral mass and THg in the foot were significantly higher in the reference pond than in the experimental pond.

All of the increase in THg in the foot of mussels in reference and preflooded experimental ponds above July 1992 background concentrations were attributable to increases in MeHg. For other body parts, MeHg contributed about 35 to 45% to the increased

Table 4. Concentrations of MeHg and THg (ng/g dry weight) and their ratio in body parts of mussels freshly collected from the source lake. The contribution of the MeHg in each body part to the whole body MeHg burden is given. Values are means ± SE. Numbers of samples analyzed are the same as no. of mussels in Table 2, except as indicated

Sampling date	Parameter	Mantle	Gill	Foot	Kidney	Visceral remains	Composite whole body
July 1992	MeHg	108 ± 9	171 ± 19[a]	250 ± 18	288 ± 23[b]	151 ± 9	161 ± 9
	THg	245 ± 14	315 ± 44	422 ± 33	1,992 ± 250	322 ± 14	368 ± 18
	MeHg : Hg (%)	45.1	57.5	61.7	14.9	47.0	43.9 ± 1.7
	MeHg as % body burden	5.2 ± 0.6	20.2 ± 1.7	9.7 ± 0.7	5.9 ± 0.4	59.0 ± 2.2	100
July 1993	MeHg	154 ± 8	269 ± 28[c]	355 ± 29	618 ± 37[d]	213 ± 13	227 ± 31
	THg	260 ± 24	368 ± 47	n/s[e]	2,697 ± 473	415 ± 22	n/d[f]
	MeHg : Hg (%)	62.8	67.8	n/d	35.5	52.0	n/d
Oct. 1993	MeHg	224 ± 16	137 ± 12	422 ± 27	482 ± 36	203 ± 16	208 ± 41
	THg	343 ± 18	256 ± 51	458 ± 24	2,260 ± 167	478 ± 35	340 ± 29
	MeHg : Hg (%)	65.8	64.5	92.6	21.5	42.7	63.4 ± 4.6
	MeHg as % body burden	8.5 ± 1.1	31.5 ± 5.4	11.3 ± 0.8	6.1 ± 0.5	42.7 ± 4.2	100

[a] $n = 11$.
[b] $n = 6$.
[c] $n = 5$.
[d] $n = 4$.
[e] n/s = insufficient sample mass for this analysis.
[f] n/d = no data.

THg in mantle and visceral remains, a variable amount (< 1–43%) in gill, and about 10% in kidney.

Changes in MeHg and THg in mussels exposed to wetland flooding

In October 1993, levels of MeHg and THg in the mantle, foot, and visceral remains of mussels from the experimental pond were significantly higher than those from the reference pond (Figs. 1 to 3). Methyl Hg and THg in mantle and visceral remains were about 104% and 39% higher, respectively, than those from the reference pond at the same time (Figs. 1 and 3), whereas the respective differences for foot were 47% and 10% (Fig. 2). The effect of flooding as judged by the magnitude of the interaction term in the two-way ANOVA on site and year was highly significant for MeHg (mantle, $p = 0.0001$; foot, $p = 0.0001$; visceral remains, $p = 0.0001$), for THg (mantle, $p = 0.007$; foot, $p = 0.019$; visceral remains, $p = 0.0001$), and for the ratio MeHg : THg (mantle, $p = 0.019$; foot, $p = 0.016$; visceral remains, $p = 0.007$). Methyl Hg, THg, and MeHg : THg in gill and kidney increased with flooding, but not significantly (data not shown).

Mantle, foot, and visceral remains of mussels in the experimental pond in October 1993 had higher MeHg and THg levels than at that site in October 1992. For example, MeHg and THg in visceral remains were 142 and 59% higher, respectively. In contrast, these metals in visceral remains of mussels in the reference pond showed little year-to-year difference; MeHg was 2% lower, THg 12% lower, and MeHg : THg 12% higher in October 1993 compared with October 1992.

The increase in THg concentrations in mussels subject to flooding (October 1993) above values in fresh mussels in July 1993 in the visceral remains was entirely accounted for by the increase in concentration of MeHg. Methyl Hg accounted for 42 and 54% of the increases in THg in the mantle and kidney, respectively.

Unexpectedly, the mussels in the flooded pond from July to August 1993 showed significant losses of MeHg and THg in all body parts and the composite body and a reduction in the ratio of MeHg : THg compared to all other caged or fresh mussels in the experiment. Concentrations of MeHg, as ng/g dry weight, were mantle, 59.5; gill, 51.8; foot, 49.0; kidney, 100.5, and visceral remains, 64.8. Corresponding concentrations for THg were mantle, 223.3; gill, 266.7; foot, 172.8; kidney, 1139.7; and visceral remains, 272.0.

DISCUSSION

This study demonstrates that unionid mussels, *P. grandis*, were effective monitors not only of large increases in MeHg

Table 5. MeHg and THg concentrations in mussels transplanted in 1992 to the reference pond and experimental pond compared to source lake mussels freshly collected in July 1992. Values are means

		Reference pond		Experimental pond	
Body part		August	October	August	October
Mantle	MeHg	172.6***	211.9***	154.3**	180.1***
	THg	378.3***	489.7***	365.1**	426.8***
Foot	MeHg	306.1	371.8***	316.7	275.7
	THg	442.6	535.9*	400.6	438.3
Kidney	MeHg	301.6	382.6**	332.2	376.4
	THg	2,548.0	3,430.0	2,635.0	2,698.0
Viscera	MeHg	183.3	245.4***	158.8	207.8***
	THg	532.6***	578.5***	377.7	443.3**

Probability that the means are not different from those of July 1992 mussels: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

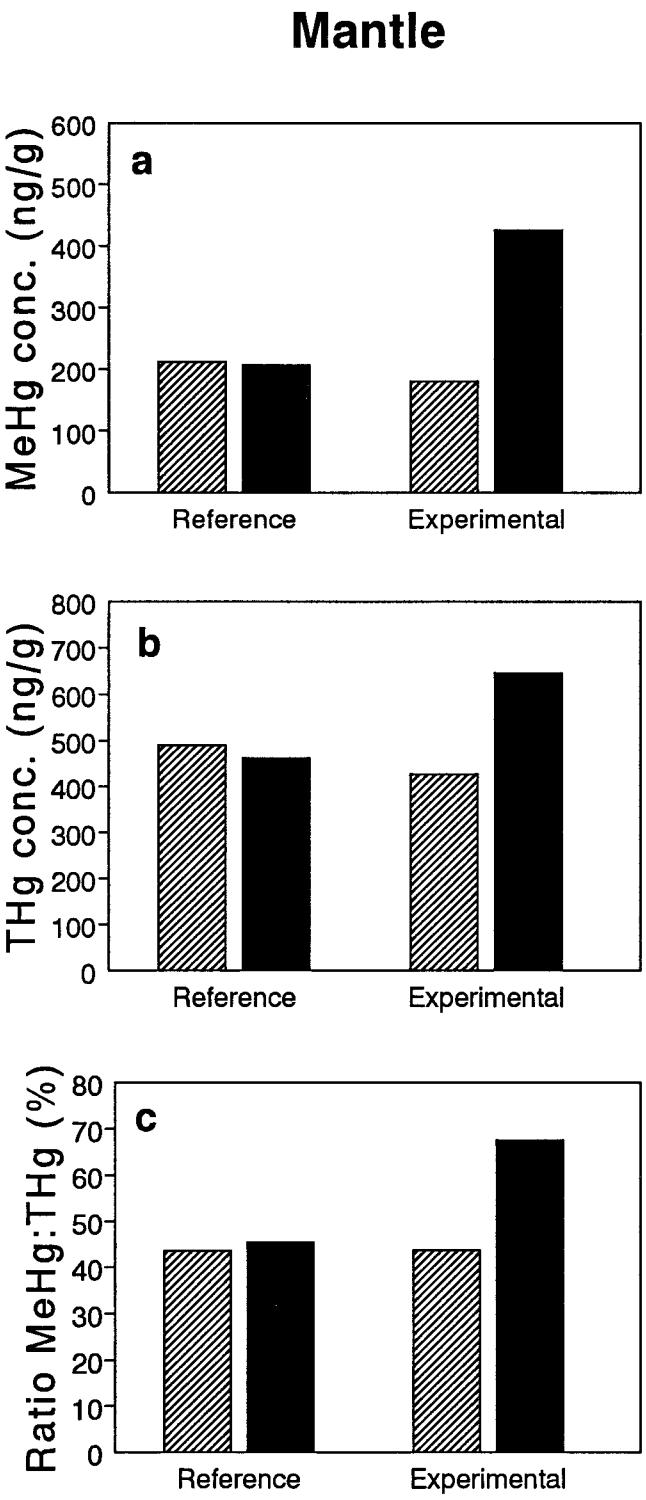

Fig. 1. Mean MeHg (**a**) and THg (**b**) concentration and the ratio MeHg : THg (**c**) in the mantle of mussels caged in the reference or experimental pond in October 1992 (hatched bars) and October 1993 (solid bars).

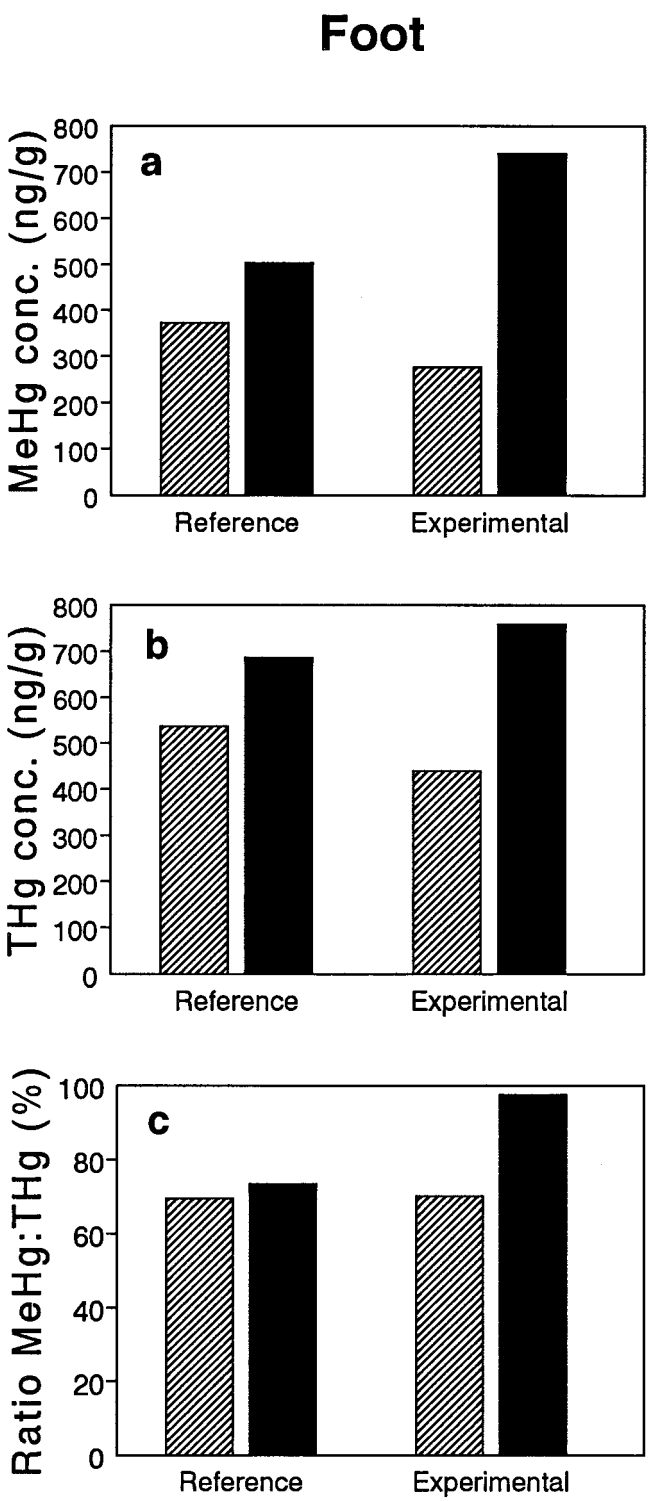

Fig. 2. Mean MeHg (**a**) and THg (**b**) concentration and the ratio MeHg : THg (**c**) in the foot of mussels caged in the reference or experimental pond in October 1992 (hatched bars) and October 1993 (solid bars).

associated with wetland flooding but also were sufficiently sensitive to detect small differences in natural levels of waterborne MeHg between different types of wetlands. Mussels transplanted from the source lake to the reference pond for 90 d showed a 63% increase in MeHg in visceral remains above pretransplant levels compared with a 38% increase in those transplanted to the preflooded experimental pond. The higher MeHg values in the reference pond mussels corresponds with the relative differences in the two ponds in levels of waterborne MeHg and THg. Compared with the preflooded experimental pond, the reference pond had naturally higher concentrations of THg (3.3 vs. 2.6 ng/L) and MeHg (0.24 vs. 0.09 ng/L) in the water [16]. As reported by the ELARP researchers, the experimental pond receives water primarily from upstream Lake 240, which has

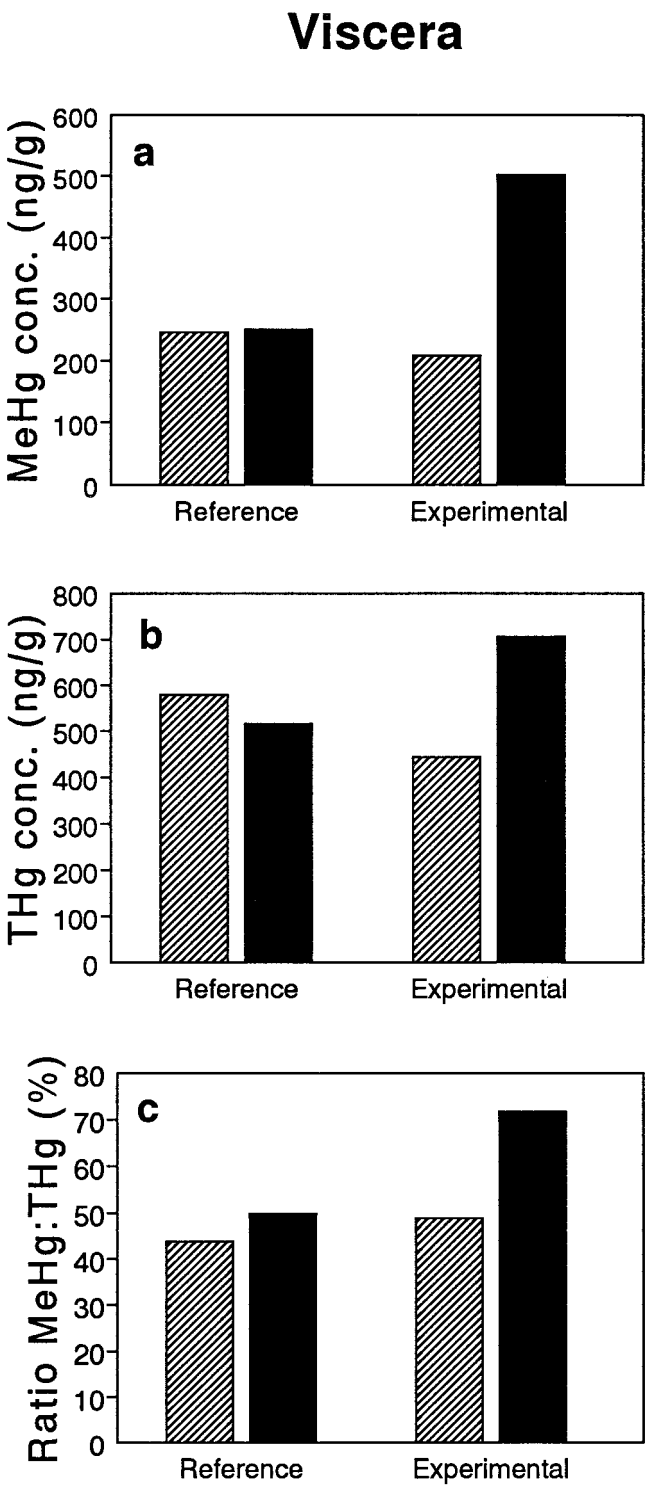

Fig. 3. Mean MeHg (**a**) and THg (**b**) concentration and the ratio MeHg : THg (**c**) in the visceral remains of mussels caged in the reference or experimental pond in October 1992 (hatched bars) and October 1993 (solid bars).

an upland terrain catchment. In contrast, the reference pond was within a headwater wetland and received water with higher MeHg concentrations than did the experimental pond. Catchments that contain wetlands yield higher amounts of MeHg than do upland catchments [22]. Higher MeHg concentrations in mussels in the reference pond than in the experimental pond were consistent with findings for other biota in the two systems. The biota (e.g., introduced fish) of the reference pond tended to have higher MeHg concentrations than those in the preflooded pond [23].

The relative increases in THg in the transplanted mussels in 1992 also reflected the differences between the two ponds in waterborne THg. The difference between ponds in waterborne THg was proportionately less than for MeHg. Total Hg in the reference pond was only 1.27-fold higher than in the experimental pond compared with a 2.7-fold difference for MeHg [16]. The mussels in the reference pond had THg in visceral remains 80% above pretransplant levels and 30% above in those in the experimental pond.

Given the sensitivity of mussels to small differences in waterborne MeHg, it is not surprising that mussels reflected the impact of flooding of the experimental pond in 1993. Flooding caused a large increase in the rate of Hg methylation resulting in a 20-fold increase in waterborne MeHg [16]. The mussels caged in the flooded pond for 90 d in 1993 reflected the increase in the water MeHg concentrations by a 140% increase above preflood levels in MeHg in the visceral remains. The increase in MeHg concentrations in mussels in response to the flooding was comparable to that found in other aquatic organisms in the experimental pond. Predacious insects increased two and one half to three times in MeHg from 50 to 80 ng/g dry weight before flooding to 200 to 350 ng/g dry weight after flooding (B.D. Hall, unpublished data). Although absolute concentrations of THg in finescale dace (*Phoxinus neogaeus*) were much higher than those in macroinvertebrates, the fish also experienced about a two times increase in response to flooding [23]. Zooplankton populations in the flooded experimental pond increased in MeHg concentrations by four to five times (M. Paterson, unpublished data).

Other studies examining the effect of lake manipulation on MeHg concentrations in macroinvertebrates report similar trends, although the magnitude of the increases differs (MeHg increases of 12 times [24] and 7 times [25] for insects and 1.3 times for zooplankton [25] from impoundments compared to natural lakes.

The distributions of MeHg and THg among body parts (mantle < gill or visceral remains < foot, kidney) generally agree with results from other field studies. The distribution of Hg in *P. grandis* body parts collected from Clay lake, Ontario, were mantle < gill < foot < visceral remains < liver < adductor muscle [7]. Laboratory-exposed mussels generally have higher concentrations in gill and mantle than field-exposed mussels. *Pyganodon grandis* exposed to Hg (10, 50, and 100 μg/L) for 2 weeks accumulated more Hg in the gill and liver than in the foot [7]. *Anodonta cygnea* exposed in the laboratory to 10 μg/L (as $HgCl_2$) for 840 h accumulated Hg in the order adductor muscles < foot < gill < mantle < kidney [8]. The difference in Hg distribution among body parts from field or laboratory studies may be related to the length of exposure, route of Hg uptake, and redistribution among body parts. In fish, MeHg entering from the gut or gills is redistributed to muscular tissues where it accumulates, bound to sulfhydryl groups in protein [26].

The relative importance of the two routes of MeHg uptake, food and water, has been examined in fish in the laboratory [27,28], as well as through Hg uptake and bioenergetics models [29,30]. A field experiment using natural levels of MeHg in both food and water confirmed past studies by demonstrating that the majority of Hg in fish tissue comes in via food [31]. Parks et al. [32] report a similar conclusion based on a study

on routes of MeHg uptake in crayfish held in the contaminated Clay Lake, Ontario, system. The primary route of contaminant uptake by mussels is not clear. King and Davies [33] suggest that for the marine mussel *Mytilus edulis* L., uptake routes change from water to particulates depending on the concentration of suspended particles. In the laboratory, they found that the accumulation of inorganic Hg from water (1 μg/L) was 10 times higher than that from suspended sediment (1 μg/L), but under estuarine conditions when the relative concentration of Hg on suspended sediments was 10 to 80 times higher than the dissolved Hg concentration, accumulation from the particulate pathway was dominant. In the present experiment, after flooding there was a dramatic increase in suspended particulates in the pond, comprised of decaying peat and vegetation, and bacteria feeding on the decaying material. Because MeHg is predominantly bound to particulates, it is possible that these particles were the primary source of MeHg to the mussels.

The loss of MeHg and THg from the visceral remains of mussels caged at the hypoxic site was unexpected. The six of eight surviving mussels exposed to flooded conditions for 48 d showed no overt signs of stress, yet had only 20 to 30% and 41 to 91% of the July 1993 pretransplant levels of MeHg and THg, respectively. It is likely that the surviving mussels spent much of the time in this hypoxic environment with the valves closed, thus reducing their opportunity to take up MeHg. It is known that fish can depurate MeHg in the laboratory [34] or in the field, if MeHg-contaminated fish are transferred from a contaminated environment to a relatively uncontaminated one [35]. The cause of the decline of MeHg in all body parts to levels well below background while the mussels were exposed to the elevated postflood waterborne MeHg is not understood. Growth dilution is ruled out because there was essentially no growth in these mussels. The lability of MeHg in these mussels caged in a hypoxic environment suggests further work should be done on environmental conditions that may lead to reduction in tissue concentrations and body burdens of MeHg in fish and other aquatic organisms.

There is no evidence that the act of caging affected condition or MeHg or THg concentrations in body parts. Mussels caged for about 90 d in the source lake or the reference or experimental ponds changed little in length. They either did not gain weight or lost small amounts of weight. Therefore, condition was marginally poorer after caging. Caging did not result in gross signs of deterioration of mussel health or unseasonal expulsion of glochidia. Caged mussels were assumed to be physiologically unimpaired in terms of their ability to take up Hg. Caging did not appear to influence the bioavailability of MeHg via the increased surface area offered by the cage materials. These results support the validity of caging mussels which is important in their use as biomonitors.

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