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EVIDENCE OF IMPAIRED HEALTH IN YELLOW PERCH (*PERCA FLAVESCENS*) FROM A BIOLOGICAL MERCURY HOTSPOT IN NORTHEASTERN NORTH AMERICA

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Abstract—Few studies have investigated the effects of mercury (Hg) on wild fish from remote areas, even though these fish can have high total Hg concentrations. In Kejimkujik National Park and National Historic Site (KNPNHS), Nova Scotia, Canada, concentrations of total Hg in many yellow perch (*Perca flavescens*) currently exceed the estimated threshold level for adverse effects in fish ($0.2\ \mu g\ Hg\ g^{-1}$ (wet wt), whole body). To determine whether Hg exposure is adversely affecting the general health of these fish, the authors collected male and female perch in the fall of 2009 and 2010 from 12 lakes within KNPNHS. The health endpoints condition, liver somatic index (LSI), and macrophage aggregates (MAs; indicators of oxidative stress and tissue damage) in the liver, kidney, and spleen were examined, and in female perch were compared between lakes and related to Hg concentrations measured in the muscle and liver tissue. No negative relationships between fish condition or LSI and Hg were found. However, within the liver, kidney, and spleen tissues of females, the relative area occupied by MAs was positively related to both muscle and liver Hg concentrations, indicating the health of these perch was adversely affected at the cellular level. These findings raise concerns for the health of these perch as well as for other wild fish populations known to have similarly elevated Hg concentrations. Environ. Toxicol. Chem. 2013;32:627–637.

Keywords—Ecotoxicology Macrophage aggregates Methylmercury Yellow perch

INTRODUCTION

The contamination of aquatic ecosystems with mercury (Hg), and consequently its major transformation product methyl mercury (MeHg), is a global concern due to its anthropogenic release and long-range transport and deposition. Methyl mercury is the dominant form of Hg found in whole wild fish [1], and through the process of biomagnification can accumulate in their tissues to concentrations that may adversely affect various aspects of their physiology and behavior. In the laboratory, the chronic dietary exposure of fish to MeHg has resulted in decreased growth [2], reduced swimming activity [3], and tissue damage in the liver and kidney (necrotic areas and increased macrophage aggregates) [4], as well as changes at the molecular level including gene expression [5] and decreased antioxidant enzyme activity [3].

Although the toxicity of Hg has been demonstrated in laboratory studies, the effects of MeHg on fish in their natural environment have been much less studied. Moreover, the majority of studies that do report MeHg effects on wild fish focus on areas with direct industrial contamination [6,7], or rivers and reservoirs impacted by human actions [8–10]. Throughout North America, however, many fish populations inhabit aquatic systems that are remote from human activities, but have whole-body MeHg concentrations (e.g., [11,12]) that exceed the $0.2\ \mu g\ g^{-1}$ wet weight (whole body) threshold that has been proposed to be protective of fish health [13]. The few studies that have been conducted in remote systems indicate that MeHg may be affecting the health of these fish, including reports of decreased condition and increased liver color (due to increased lipofuscin, a pigment resulting from lipid peroxidation) [14], kidney and spleen damage (measured by macrophage aggregates; MAs) [15], and decreased liver size [16].

Kejimkujik National Park and National Historic Site (KNPNHS) in Nova Scotia, Canada, is relatively remote and does not have any inputs of industrial point source pollution to the ecosystem [17]. Even so, the region has been designated a biological mercury hotspot based on the Hg concentrations present in yellow perch (*Perca flavescens*) and common loons (*Gavia immer*) within the park [18]. Furthermore, total Hg (THg; the sum of all forms of Hg) concentrations in whole yellow perch within KNPNHS have increased an average of 29% in 10 of 16 lakes since 1996 to 1997, and 13 of these lakes contain yellow perch with mean THg (>94% MeHg [1]) concentrations that exceed the $0.2\ \mu g\ g^{-1}$ wet weight threshold [11]. The objective of the present study was to determine whether MeHg exposure is adversely affecting the general health of wild yellow perch within KNPNHS. To achieve this, perch were sampled across a range of known mean Hg concentrations [11], and the health endpoints condition, liver somatic index (LSI), and MAs in the liver, kidney, and spleen were measured. These endpoints measure perch health at the whole-body, organ, and cellular levels, respectively. Both condition and LSI are measures of energy storage within a fish, and may be reduced due to increased energy demands for processes such as metal detoxification. The MAs are groups of phagocytic white blood cells that can form in fish tissues and that can function as metabolic depository sites for cellular

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debris [19]. Their size and number therefore increase with increased tissue degeneration or cellular damage [19]. In addition, tissues within and around MAs in liver were compared for Hg concentrations to determine whether there were links between the presence of this metal and tissue damage.

MATERIALS AND METHODS

Study site and sample collection

Located in southwestern Nova Scotia, Canada, KNPNHS is primarily used for tourism and outdoor recreation. Lakes within KNPNHS tend to be shallow, oligotrophic, and acidic (Table 1), and sexually mature and immature male and female yellow perch were captured from 12 lakes within the park (Table 1). The majority of perch were caught using fyke and trap nets set overnight in shallow littoral zones of the lakes, and angling was also used to catch larger perch. This combination of methods allowed the capture of perch of varying sizes from different areas within the lakes. Both male and female perch were then randomly selected for dissection from the perch caught within each lake, while all others were immediately released. Sampling occurred in late September 2009 and 2010; six lakes were sampled in 2009, nine lakes were sampled in 2010, and three of the lakes (Big Dam East, Peskowesk, and North Cranberry) were sampled in both 2009 and 2010. The fork length ($\pm$ 1 mm) and total weight ($\pm$ 0.1 g) of each perch were measured before fish were euthanized by spinal severance and dissected according to procedures approved by the University of New Brunswick Animal Care Committee (Canada). A dorsal muscle fillet was collected above the lateral line of each perch and frozen for Hg analysis. The whole liver from each fish was weighed ($\pm$ 0.001 g), a portion was preserved for histology (2009 only, six lakes representing a range in Hg concentrations), and an additional portion was frozen for Hg analysis. In 2009, whole kidney and spleen tissues were collected from most perch from six lakes (as above) for histology, but in some cases were too small for analysis. Tissue samples for histology were immediately placed in 10% phosphate-buffered formalin. For age determination, scales were removed from directly behind the pectoral fin, and ages were estimated as described by Devries and Frie [20]. Body condition factor (K) was calculated as $(100 \times (\text{total weight } \{g\}/\text{fork length}^3 \{cm\}))$, and LSI was calculated as $(100 \times (\text{liver weight } \{g\}/\text{total weight } \{g\}))$.

Mercury analysis

Liver and muscle tissue samples were lyophilized using a Labconco Freezone 12 and ground with a glass rod prior to Hg analysis. The moisture content in each tissue sample was determined, and all results are presented as wet-weight concentrations. Kidney and spleen tissues were not collected or analyzed for Hg content due to their small size. The distribution of Hg in fish tissues is variable, but the Hg concentrations found in kidney and spleen tissues following dietary MeHg exposure can be relatively similar to those found in the liver [21]. Therefore, health endpoints measured in the kidney and spleen were related to liver Hg concentrations, although the use of Hg concentrations from these other two tissues would have been ideal.

In all muscle samples, THg concentrations were measured using a Milestone DMA-80 Direct Mercury Analyzer at the University of New Brunswick, Saint John, Canada. To ensure quality control, blanks were analyzed, as well as a 15-ng liquid Hg standard, standard reference materials TORT-2 and DORM-2 (lobster hepatopancreas and dogfish muscle), from the National Research Council of Canada, and an intralaboratory standard (individual yellow perch muscle sample). Mean recoveries of these were 85.6% $\pm$ 0.8 ($n = 65$) for the liquid standard, 107.8% $\pm$ 1.0 for TORT-2 ($n = 63$), and 91.7% $\pm$ 0.8 ($n = 44$) for DORM-2. The mean concentration of blanks was 0.0087 $\mu g\, g^{-1}$ Hg ($n = 97$) based on sample weight of 0.0100 g, and the relative percentage difference between sample duplicates was 2.6% $\pm$ 0.5 ($n = 42$). The coefficient of variation of the intralaboratory standard was 3.2% ($n = 63$).

In liver samples, and in a subset of muscle samples, MeHg and inorganic Hg (HgII) concentrations were measured using a Brooks Rand Model II system at Acadia University, Nova Scotia, Canada. Samples were digested prior to analysis using methods similar to those of Cai and Bayona [22]. Weighed samples were digested in 25% KOH/MeOH solution, shaken for 1 h, and heated for 1 h at 95°C. Analyses were then conducted according to U.S. Environmental Protection Agency Method 1630 [23], through aqueous ethylation, purge and trap gas chromatography, and detection using atomic fluorescence spectrometry. The THg concentrations were calculated from these analyses as the sum of all measured Hg concentrations within a sample, using separate HgII and MeHg standard curves to quantify the respective Hg species. All muscle THg data

Table 1. Physical characteristics of study lakes within Kejimkujik National Park and National Historic Site, Nova Scotia[a]

Lake	Surface area (Ha)[b]	Max. depth (m)[b]	pH[c]	Total organic carbon (mg L^{-1})[c]	Coordinates
Upper Silver	24	5.8	6.32 $\pm$ 0.01	3.60 $\pm$ 0.15	44°17′ 7.10″N, 65°14′ 54.71″W
Big Dam East	46	4.2	6.25 $\pm$ 0.03	4.45 $\pm$ 0.41	44°26′50.03″N, 65°15′ 40.91″W
Beaverskin	40	6.2	5.81 $\pm$ 0.02	2.82 $\pm$ 0.17	44°18′ 25.50″N, 65°19′ 58.29″W
Cobrielle	132	6.2	5.62 $\pm$ 0.07	3.05 $\pm$ 0.21	44°18′ 50.48″N, 65°14′ 9.76″W
Mountain	136	14.2	5.37 $\pm$ 0.07	4.20 $\pm$ 0.12	44°19′ 22.76″N, 65°15′ 5.29″W
Grafton	270	10.0	6.17 $\pm$ 0.02	6.43 $\pm$ 0.89	44°23′ 1.05″N, 65°11′ 5.29″W
Big Dam West	105	9.5	5.10 $\pm$ 0.09	12.63 $\pm$ 2.72	44°27′ 38.22″N, 65°17′ 27.51″W
Hilchemakaar	95	7.2	5.83 $\pm$ 0.08	5.68 $\pm$ 0.29	44°17′ 33.86″N, 65°14′ 42.36″W
George	78	8.5	5.11 $\pm$ 0.05	9.63 $\pm$ 1.12	44°20′ 23.10″N, 65°12′ 39.69″W
Kejimkujik	2,435	19.2	5.14 $\pm$ 0.03	9.78 $\pm$ 1.04	44°22′ 56.19″N, 65°14′ 16.86″W
North Cranberry	34	5.0	5.50 $\pm$ 0.03	4.33 $\pm$ 0.12	44°19′ 54.26″N, 65°13′ 59.71″W
Peskowesk	685	13.0	4.99 $\pm$ 0.09	6.90 $\pm$ 0.53	44°18′ 48.27″N, 65°17′ 4.34″W

[a] Mean values ($\pm$ SE, $n = 4$) are presented for pH and total organic carbon.
[b] Data taken from Drysdale et al. [46].
[c] Data collected twice per year in 2009 and 2010 by Environment Canada.

measured using this method were used only for determining the percentage of MeHg content in this tissue (see *Results* section). Quality control measures included the standard reference materials DORM-2 and DOLT-4 (dogfish liver) from the National Research Council of Canada, sample duplicates (duplicated through the digestion process), and triplicate analysis of selected digested samples. Mean percentage of recoveries of MeHg and HgII for DOLT-4 were 105.2 ± 0.16 and 96.8 ± 1.6 ($n = 31$), and for DORM-2 were 115.5 ± 4.4 and 167.9 ± 6.6 ($n = 4$), respectively. The duplicate relative percentage of differences of MeHg and HgII, respectively, were $11.8\% \pm 2.7$ ($n = 24$) and $16.2\% \pm 4.5$ ($n = 24$), and the triplicate relative percentage of differences for these were $5.9\% \pm 1.1$ ($n = 13$) and $6.1\% \pm 0.9$ ($n = 14$). The limit of detection for the analyses was $0.0002\ \mu g\ g^{-1}$ for MeHg and $0.0013\ \mu g\ g^{-1}$ for HgII, and was calculated as three times the standard deviation of the respective method blanks. The relative percentage of difference between muscle THg concentrations measured at Acadia University, and those measured at the University of New Brunswick, was $25.5\% \pm 3.8$ ($n = 40$).

Macrophage aggregate analysis

Liver, kidney, and spleen samples were dehydrated in ethanol and toluene, embedded in paraffin, sectioned to 8 μm, mounted on slides, and stained with hematoxylin and eosin. Prior to embedding, samples were given new, random sample numbers to prevent bias during analysis. All slides were assigned randomly to staining batches, and the same staining protocol and dyes were used for all batches.

Macrophage aggregates were quantified within the prepared tissues using methods similar to those of Drevnick et al. [14]. One stained slide containing several tissue sections was prepared for each sample. Three tissue sections were selected per slide, and a representative area of each section was photographed at 70× magnification using a Leica DM 2500 microscope equipped with a Leica DFC 290 camera (Fig. 1). Each photograph was then overlaid with a grid of squares (50×50 μm) using Adobe Photoshop CS5 software, and 100 squares (out of 266) were selected using a random number generator. For each selected square, the presence or absence of MAs was recorded. The proportion of squares containing MAs per 100 selected squares was calculated for each photograph, and this process was completed for each of the three photographs taken per sample. The averaged proportion from the three photographs for each tissue type, a measure of the relative area occupied by MAs within a tissue, was used for data analysis.

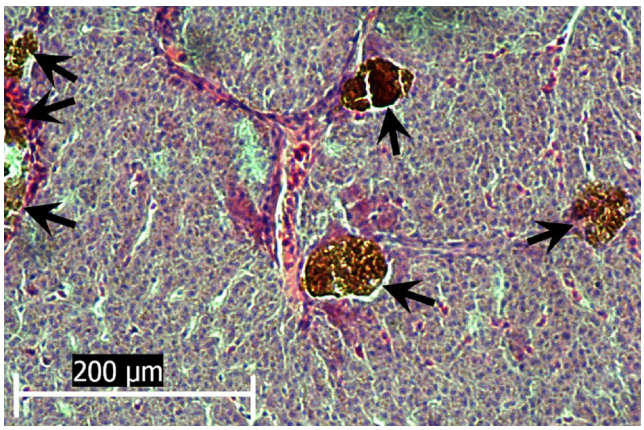

Fig. 1. Liver tissue section from a mature yellow perch photographed at 70× magnification and stained with hematoxylin and eosin. Black arrows indicate macrophage aggregates. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com.]

Some small tissue pieces did not fill the entire field of view of the photographs. For these, randomly selected squares containing <50% tissue coverage were not included in the MA count. The relative tissue area occupied by MAs within these slides was therefore calculated as *(number of squares counted with MAs present)/(100–number of squares with <50% tissue coverage)*.

Laser ablation inductively coupled–mass spectrometry

The distribution of mercury in yellow perch liver sections was examined using laser ablation inductively coupled–mass spectrometry (LA ICP–MS) as described by Barst et al. [24]. Briefly, an uncoverslipped microscope slide, with Prussian Blue–stained liver tissue sections (40-μm thick; processed as described above), was placed into the chamber of a 213-nm Nd:YAG laser ablation source (New Wave Research). Tissues were stained prior to analysis using a Sigma Aldrich Iron Stain Kit HT20 according to kit procedures, with the exception that the staining time in the working iron stain solution was reduced to 5 s and pararosaniline counterstain was not used. A digital camera allowed zooming and scanning of the slide to locate macrophage aggregates.

Four to five areas of macrophages and adjacent equally sized areas of hepatocytes were chosen at random and ablated from liver sections from 10 individual perch. Perch were selected so as to reflect a range of THg concentrations. A 65-μm-diameter round spot allowed adequate sampling of MA and decreased the risk of interferences and resampling of previously ablated material. A Varian 820 ICPMS coupled to the laser source was used to monitor 202 Hg isotopes. The mass detector collected data for 70 s each run, the first 10 s of which was laser warm-up. Mean isotope counts for the first 10 s were used as an estimate of background noise and subtracted from the mean counts for the remainder of the run to calculate a signal.

Statistical analyses

To determine whether the health endpoints (condition, LSI, and MAs) differed with tissue Hg concentrations in perch, these endpoints were related to the tissue Hg concentrations and were also compared among lakes. Analyses were performed using Systat 11 (Systat Software), and the level of statistical significance was set at $p < 0.05$. All results are presented as mean ± standard error. Data were transformed to approximate the homogeneity of variance and normality assumptions of the analyses. Length, weight, age, condition, LSI, and Hg concentration data were log transformed, and MA data were arcsine square root transformed. In some cases these assumptions were not met; however, analysis of variance and analysis of covariance are robust to fairly severe violations of these assumptions [25].

In lakes that were sampled in both 2009 and 2010, LSI and condition data from the two years were combined within each lake and within each sex because these data did not differ between the sampling years using two-sample *t* tests (Condition: $p > 0.268$, LSI: $p > 0.106$). For sample sizes less than three, these data were combined without a priori statistical analyses. Two-sample *t* tests were also used to determine sex differences in tissue MAs (using pooled data from all the lakes), and a paired *t* test was used to compare the mean 202 Hg isotope signal between hepatocytes and MAs for LA ICP–MS

data. Due to small sample sizes, further analysis of male perch data was limited to the comparison of MAs between sexes described below. Analysis of variance and Tukey's post hoc test were used to determine differences between lakes in female condition, LSI, length, tissue MAs, and muscle and liver Hg concentrations. For more details of these analyses for MA data, see Supplemental Data, *Statistical analyses* section.

Using pooled data from all lakes, least squares linear regression was used to determine significant relationships between tissue Hg concentrations, and condition, LSI, length, age, or the relative area occupied by MAs within liver, kidney, and spleen tissues. Only sample sizes of nine or greater were used for linear regressions, and studentized residuals with an absolute value >3 were considered outliers and omitted from the analyses. For regressions with the health endpoints condition, LSI, and tissue MAs, regressions were conducted for three size classes of perch: ≤10 cm, >10 cm, and all lengths. These classes were created based on the findings of Wyn et al. [11] to investigate whether Hg affected smaller perch (≤10 cm) differently from larger perch. To determine age-independent relationships between tissue MAs and Hg concentrations, residuals of the linear regression between age and tissue MAs (for females of all lengths) were used to remove age as a confounding variable. Length classes (≤ and >10 cm) were also used, similar to the age class approach of Schwindt et al. [15].

Analysis of covariance was used to compare the slopes and intercepts of the linear relationships between age and tissue MAs among lakes, as well as the relationships between MAs and age, and between MAs and liver (THg) among tissue types. Similarly, analysis of covariance was also used to compare the linear relationships between liver MAs and liver MeHg concentrations between sexes (using male and female perch of the same age ≤2 y old), to compare the relationships between muscle (THg) and female perch length among lakes, and to compare the relationships between muscle (THg) and length or age between the sexes.

RESULTS

Tissue mercury concentrations

Muscle THg concentrations in all perch ranged from 0.08 to 2.13 μg g^{-1}, and MeHg comprised 96.5% ± 0.3 ($n = 40$) of the measured THg in muscle tissue (sexes combined). Within females, the muscle THg concentrations differed significantly ($F_{11,246} = 4.713$, $p < 0.001$) among lakes, with the highest mean THg concentration more than twofold higher than the lowest (Table 2). Muscle (THg) was positively related to the length of male (M) and female (F) perch (F: $F_{1,253} = 212.533$, $p < 0.001$, $r^2 = 0.457$; M: $F_{1,88} = 50.785$, $p < 0.001$, $r^2 = 0.366$) as well as their age (F: $F_{1,226} = 122.346$, $p < 0.001$, $r^2 = 0.351$; M: $F_{1,74} = 4.669$, $p = 0.034$, $r^2 = 0.059$). The slopes of these relationships did not differ between the sexes when age was the independent variable ($F_{1,300} = 1.266$, $p = 0.261$, $r^2 = 0.301$), but did differ with length as the independent variable ($F_{1,341} = 9.428$, $p = 0.002$, $r^2 = 0.446$). The fork length of female perch did not differ among lakes ($F_{11,244} = 1.217$, $p = 0.276$), nor did the slopes of the relationships between length and muscle (THg) in females ($F_{1,231} = 0.953$, $p = 0.490$, $r^2 = 0.600$). In addition, mean muscle THg concentrations in perch from each lake (sexes combined) were negatively related to mean lake pH ($F_{1,10} = 7.796$, $p = 0.019$, $r^2 = 0.438$).

Liver THg concentrations in all perch ranged from 0.13 to 6.04 μg g^{-1} and, within females, differed significantly among lakes with means differing by up to nine fold (Table 2). These concentrations were positively related to the length of both sexes (females: $F_{1,176} = 51.691$, $p < 0.001$, $r^2 = 0.227$; males: $F_{1,35} = 5.231$, $p = 0.029$, $r^2 = 0.137$) as well as the age of females ($F_{1,170} = 70.647$, $p < 0.001$, $r^2 = 0.294$). Liver THg is comprised of HgII and MeHg, and the concentrations of these two Hg species differed among lakes (Supplemental Data, Table S1). Although concentrations of both HgII and MeHg increased with increasing THg concentrations in liver (Supplemental Data, Fig. S1), the percentage present as MeHg decreased with higher THg in this organ (Fig. 2). The variability of liver MeHg concentrations, however, also increased with increasing liver THg concentrations.

In general, THg concentrations in liver tissue were higher than those in muscle tissue, and in some individuals exceeded the muscle THg concentration by up to 12.5-fold. The ratio of these concentrations (liver:muscle THg) increased with liver (THg), and also showed a negative trend with liver % MeHg (Supplemental Data, Fig. S2A and B).

Macrophage aggregates

Among lakes, the mean relative area occupied by MAs within liver, kidney, and spleen tissues of female perch varied approximately 2.5- to 3-fold within tissue types (Supplemental Data, Table S1). The relative area occupied by MAs in female liver and spleen tissues differed among lakes (liver: $F_{5,47} = 2.604$, $p = 0.037$; spleen: $F_{5,40} = 2.577$, $p = 0.041$), but in kidney tissues, a difference among lakes was not found ($F_{5,24} = 1.287$, $p = 0.302$). Specifically, the relative area occupied by MAs in the liver tissue of females from Big Dam East was lower than that measured in females from Peskowesk ($p = 0.048$), but specific pairwise differences did not exist among lakes for spleen tissue ($p > 0.05$).

The slopes of the relationships between tissue MAs and age in female tissues did not differ among lakes (liver: $F_{5,67} = 2.227$, $p = 0.062$; kidney: $F_{5,34} = 0.848$, $p = 0.525$; spleen: $F_{5,54} = 1.131$, $p = 0.355$), so data from all lakes were combined within each tissue type for analysis. The relative area occupied by MAs in all female liver, spleen, and kidney tissues was positively related to muscle (THg) and all Hg concentrations in the liver (Table 3). The linear relationships between tissue MAs and liver THg concentrations, however, differed among the three tissue types (Fig. 3A, D, and G). The slopes of these relationships were parallel ($F_{2,158} = 0.188$, $p = 0.829$), but the intercepts differed ($F_{2,160} = 62.484$, $p < 0.001$), and at a given liver (THg), the relative area occupied by MAs was highest in the spleen and lowest in the liver. This difference among tissues was also true in relation to age (Fig. 3B, E, H); the slopes of the lines were parallel ($F_{2,153} = 0.844$, $p = 0.432$); however, the intercepts differed ($F_{1,155} = 40.006$, $p < 0.001$). In addition, the relative area occupied by MAs in liver tissue was negatively related to LSI ($F_{1,77} = 20.147$, $p < 0.001$, $r^2 = 0.207$).

Negative relationships also existed between lake pH and the relative area occupied by MAs in the livers of female perch of all lengths (Supplemental Data, Table S2). In addition, for liver, pH explained a higher amount of variability in the relative area occupied by MAs ($r^2 = 0.667$) than that explained by tissue Hg concentrations (Table 3).

Age-independent macrophage aggregate relationships with Hg

In females of all lengths, residuals of the relationship between age and the relative area occupied by MAs in kidney and spleen tissue, but not liver tissue (Fig. 3C), were positively

Table 2. Summary statistics for male and female yellow perch from Kejimkujik National Park and National Historic Site, Nova Scotia[a]

Lake/group	Age (y)	Length (cm)	Weight (g)	Condition	LSI	Muscle THg ($\mu g\,g^{-1}$ wet wt)	Liver THg ($\mu g\,g^{-1}$ wet wt)
				Females			
Upper Silver	1.8 ± 0.3 (19)	11.1 ± 0.8 (19)	19.8 ± 4.9 (19)	1.06 ± 0.02 (19)BC	0.89 ± 0.04 (18)A	0.26 ± 0.02 (19)C	0.25 ± 0.03 (15)E
Big Dam East	2.6 ± 0.3 (28)	11.7 ± 0.5 (36)	19.9 ± 2.9 (37)	1.03 ± 0.02 (36)C	0.75 ± 0.04 (31)AB	0.36 ± 0.03 (37)BC	0.48 ± 0.07 (23)DE
Beaverskin	1.6 ± 0.3 (14)	11.4 ± 0.9 (14)	19.4 ± 5.8 (14)	1.00 ± 0.02 (14)C	0.78 ± 0.04 (13)AB	0.35 ± 0.05 (14)ABC	0.39 ± 0.10 (12)DE
Cobrielle	2.3 ± 0.3 (28)	11.5 ± 0.5 (28)	19.1 ± 2.3 (28)	1.06 ± 0.03 (28)BC	0.77 ± 0.04 (27)AB	0.38 ± 0.03 (28)ABC	0.46 ± 0.05 (12)CDE
Mountain	1.6 ± 0.3 (14)	10.6 ± 0.7 (14)	14.8 ± 3.5 (14)	1.03 ± 0.02 (14)BC	0.81 ± 0.04 (14)A	0.38 ± 0.05 (14)ABC	0.60 ± 0.08 (12)ABCD
Grafton	2.4 ± 0.6 (17)	12.8 ± 1.2 (16)	30.3 ± 8.4 (17)	1.08 ± 0.01 (16)BC	0.95 ± 0.06 (17)A	0.43 ± 0.08 (17)ABC	0.51 ± 0.08 (13)CDE
Big Dam West	3.8 ± 0.6 (12)	13.2 ± 1.0 (12)	35.8 ± 8.4 (12)	1.26 ± 0.03 (12)A	0.90 ± 0.05 (12)A	0.45 ± 0.05 (12)ABC	1.17 ± 0.27 (11)ABC
Hilchemakaar	3.6 ± 0.5 (22)	13.0 ± 1.0 (22)	32.8 ± 8.9 (22)	1.02 ± 0.02 (22)C	0.78 ± 0.06 (22)AB	0.51 ± 0.09 (21)ABC	0.73 ± 0.23 (17)BCD
George	3.2 ± 0.6 (12)	14.1 ± 1.2 (12)	42.6 ± 13.9 (12)	1.15 ± 0.04 (12)AB	0.81 ± 0.07 (12)AB	0.58 ± 0.16 (12)AB	1.73 ± 0.41 (12)AB
Kejimkujik	3.7 ± 0.6 (12)	13.4 ± 1.5 (12)	41.5 ± 19.4 (12)	1.10 ± 0.03 (12)BC	0.85 ± 0.07 (12)A	0.60 ± 0.15 (12)AB	2.34 ± 0.65 (12)A
North Cranberry	3.6 ± 0.4 (30)	12.5 ± 0.6 (36)	26.9 ± 4.3 (36)	1.01 ± 0.02 (36)C	0.61 ± 0.04 (35)B	0.55 ± 0.04 (36)A	0.97 ± 0.13 (21)ABC
Peskowesk	3.0 ± 0.3 (34)	11.6 ± 0.5 (35)	21.2 ± 3.2 (36)	1.08 ± 0.02 (35)BC	0.76 ± 0.03 (35)AB	0.56 ± 0.04 (36)A	1.20 ± 0.16 (19)AB
≤ 10 cm	1.1 ± 0.1 (75)	n/a	6.6 ± 0.2 (83)	1.01 ± 0.01 (83)	0.75 ± 0.03 (79)	0.30 ± 0.01 (82)	0.42 ± 0.06 (35)
> 10 cm	3.6 ± 0.1 (164)	n/a	34.5 ± 2.5 (173)	1.08 ± 0.01 (173)	0.80 ± 0.02 (166)	0.53 ± 0.02 (173)	0.98 ± 0.09 (144)
All lakes	2.8 ± 0.1 (242)[b]	12.1 ± 0.2 (256)	25.2 ± 1.8 (259)[b]	1.06 ± 0.01 (256)	0.78 ± 0.01 (248)[b]	0.45 ± 0.02 (258)[b]	0.87 ± 0.07 (179)
				Males			
Upper Silver	1.2 ± 0.4 (5)	9.6 ± 0.6 (5)	9.9 ± 1.8 (5)	1.07 ± 0.02 (5)	0.68 ± 0.06 (5)	0.37 ± 0.04 (5)	0.30 ± 0.16 (2)
Big Dam East	1.0 ± 0.2 (9)	7.5 ± 0.9 (9)	6.5 ± 0.4 (9)	1.05 ± 0.05 (8)	0.69 ± 0.07 (9)	0.26 ± 0.03 (9)	n/a
Beaverskin	2.0 ± 0.5 (7)	10.0 ± 0.8 (7)	12.3 ± 4.0 (7)	1.02 ± 0.04 (7)	0.61 ± 0.04 (7)	0.43 ± 0.05 (7)	0.53 ± 0.28 (4)
Cobrielle	0.9 ± 0.2 (13)	8.4 ± 0.3 (13)	6.6 ± 0.8 (13)	1.04 ± 0.04 (13)	0.71 ± 0.04 (9)	0.30 ± 0.04 (13)	0.87 ± 0.40 (3)
Mountain	0.8 ± 0.3 (6)	7.9 ± 0.6 (6)	5.5 ± 1.1 (6)	1.03 ± 0.02 (6)	0.77 ± 0.06 (6)	0.26 ± 0.06 (6)	0.47 ± 0.03 (2)
Grafton	0.3 ± 0.3 (3)	7.8 ± 0.6 (3)	5.8 ± 1.5 (3)	1.17 ± 0.05 (3)	1.06 ± 0.16 (3)	0.20 ± 0.05 (3)	0.34 (1)
Big Dam West	1.2 ± 0.4 (5)	8.5 ± 0.8 (5)	7.4 ± 1.4 (5)	1.12 ± 0.05 (5)	0.92 ± 0.13 (5)	0.33 ± 0.08 (5)	0.87 ± 0.18 (3)
Hilchemakaar	1.1 ± 0.1 (7)	8.2 ± 2.0 (7)	5.5 ± 0.4 (7)	1.00 ± 0.02 (7)	0.82 ± 0.10 (7)	0.35 ± 0.05 (7)	0.44 ± 0.10 (6)
George	0.8 ± 0.2 (6)	8.4 ± 0.1 (6)	6.3 ± 0.4 (6)	1.06 ± 0.03 (6)	0.69 ± 0.05 (6)	0.23 ± 0.03 (6)	0.49 (1)
Kejimkujik	2.0 ± 0.0 (6)	9.6 ± 0.4 (6)	10.2 ± 1.6 (6)	1.14 ± 0.05 (6)	0.57 ± 0.08 (6)	0.38 ± 0.05 (6)	1.01 ± 0.24 (6)
North Cranberry	1.2 ± 0.2 (13)	8.6 ± 0.2 (15)	6.0 ± 0.4 (15)	0.93 ± 0.03 (15)	0.61 ± 0.03 (15)	0.54 ± 0.07 (15)	0.93 ± 0.10 (3)
Peskowesk	1.0 ± 0.1 (11)	7.3 ± 0.7 (11)	6.1 ± 0.5 (11)	1.13 ± 0.03 (10)	0.73 ± 0.06 (11)	0.50 ± 0.05 (10)	0.87 ± 0.20 (4)

[a] Mean ± standard error (n). Values that do not share the same uppercase letter are significantly different ($p < 0.05$).
[b] Includes three perch of unknown lengths.
LSI = liver somatic index; THg = total mercury; n/a = not available.

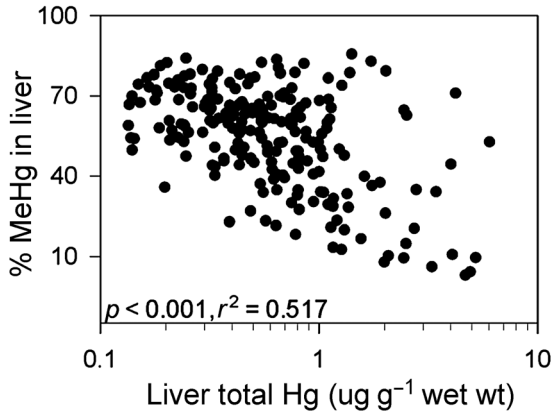

Fig. 2. Scatterplot of the relationship between percentage of liver total Hg present as methyl Hg and liver total Hg concentration (Pearson correlation coefficient shown).

related to liver inorganic Hg concentrations (Fig. 3F and I). However, in the livers of female perch ≤ 10 cm, the relative area occupied by MAs was positively related to all Hg concentrations in this tissue, with 74.5% of the variability in the occurrence of MAs explained by liver MeHg concentrations (Table 3). In females >10 cm, the measures of MAs in all three tissues were positively related to all liver and muscle Hg concentrations (Table 3), but in all tissues the strongest relationships were with liver (inorganic Hg) ($r^2 \geq 0.184$).

Sex differences in macrophage aggregate occurrence

The liver and spleen tissues of all males had a higher relative area occupied by MAs (liver: $p = 0.001$, spleen: $p = 0.010$) than those from all females of the same age. In both males and females ≤ 2 y old, the prevalence of liver MAs was positively related to liver MeHg concentrations (Fig. 4). While the slopes of these relationships were parallel ($F_{1,28} = 0.040$, $p = 0.844$), the intercept for males was higher than for females ($F_{1,29} = 6.220$, $p = 0.019$), and at a given liver MeHg concentration, the relative area occupied by MAs in liver tissue was generally higher in males than in females.

Mercury distribution in liver tissue

Forty five of 48 (94%) ablated MAs were higher in Hg isotope counts than their adjacent hepatocyte tissue ($p < 0.010$), and the mean isotope count of all ablated MAs was higher than that in all measured hepatocytes ($p = 0.005$). A mean MA:hepatocyte ratio was calculated for each fish as a relative measure of Hg accumulation in the MAs. The average MA: hepatocyte ratios ranged from 1 to 9 ($n = 10$), and the mean of all samples was 2.45 ± 0.44 ($n = 48$). Mean 202 Hg counts from MA in each fish ($n = 10$) were positively correlated with both THg in perch muscle ($F_{1,8} = 74.503$, $p < 0.001$, $r^2 = 0.903$) and liver ($F_{1,8} = 11.553$, $p = 0.009$, $r^2 = 0.591$). Mean 202 Hg counts from hepatocytes in each fish were positively correlated with THg in liver ($F_{1,8} = 8.142$, $p = 0.021$, $r^2 = 0.504$) but not in muscle ($F_{1,8} = 0.819$, $p = 0.395$, $r^2 = 0.093$).

Liver somatic index

The LSI of female perch differed significantly among lakes (Table 2), but these differences did not reflect the between-lake differences in muscle or liver Hg concentrations (Table 2 and Supplemental Data, Table S1). For all females, weak positive relationships were present between LSI and muscle THg concentrations (Table 4), as well as length ($F_{1,240} = 6.714$,

Table 3. Least squares linear regression relationships between the relative area occupied by macrophage aggregates (MAs; arcsine root transformed) in liver, kidney, and spleen tissues and tissue Hg concentrations ($\mu g\ g^{-1}$ wet wt; $\log_{10}$ transformed) in female perch of all lengths[a]

Fork length (cm)	Dependent variable	Tissue Hg species	Slope	Intercept	r^2	*P*	No.
≤10	Liver MAs	Liver total Hg	0.142	0.152	0.420	0.043	10
		Liver methyl Hg	0.210	0.241	0.745	0.003	9
		Liver inorganic Hg	0.105	0.181	0.364	0.065	10
		Muscle total Hg	0.210	0.184	0.499	0.005	14
>10	Liver MAs	Liver total Hg	0.074	0.167	0.080	0.033	57
		Liver methyl Hg	0.042	0.165	0.028	0.214	57
		Liver inorganic Hg	0.099	0.223	0.184	0.001	57
		Muscle total Hg	0.080	0.172	0.051	0.065	67
	Kidney MAs	Liver total Hg	0.130	0.292	0.138	0.039	31
		Liver methyl Hg	0.080	0.293	0.062	0.175	31
		Liver inorganic Hg	0.180	0.396	0.280	0.002	31
		Muscle total Hg	0.132	0.295	0.093	0.066	37
	Spleen MAs	Liver total Hg	0.113	0.347	0.124	0.009	54
		Liver methyl Hg	0.063	0.343	0.042	0.137	54
		Liver inorganic Hg	0.141	0.425	0.259	<0.001	54
		Muscle total Hg	0.137	0.358	0.121	0.007	60
All lengths	Liver MAs	Liver total Hg	0.101	0.168	0.152[b]	0.001	67
		Liver methyl Hg	0.073	0.173	0.082[b]	0.019	67
		Liver inorganic Hg	0.113	0.227	0.243[b]	<0.001	67
		Muscle total Hg	0.132	0.180	0.147[b]	<0.001	81
	Kidney MAs	Liver total Hg	0.165	0.304	0.235	0.002	38
		Liver methyl Hg	0.119	0.311	0.131	0.025	38
		Liver inorganic Hg	0.191	0.408	0.380	<0.001	38
		Muscle total Hg	0.192	0.311	0.215	0.001	47
	Spleen MAs	Liver total Hg	0.140	0.354	0.196	<0.001	60
		Liver methyl Hg	0.094	0.356	0.090	0.020	60
		Liver inorganic Hg	0.186	0.467	0.435	< 0.001	59
		Muscle total Hg	0.169	0.368	0.206	< 0.001	68

[a] $p < 0.05$ was considered statistically significant.
[b] r^2 values are lower than those from the equivalent significant linear relationship between the mean proportion of tissue MAs for each lake and mean lake pH.

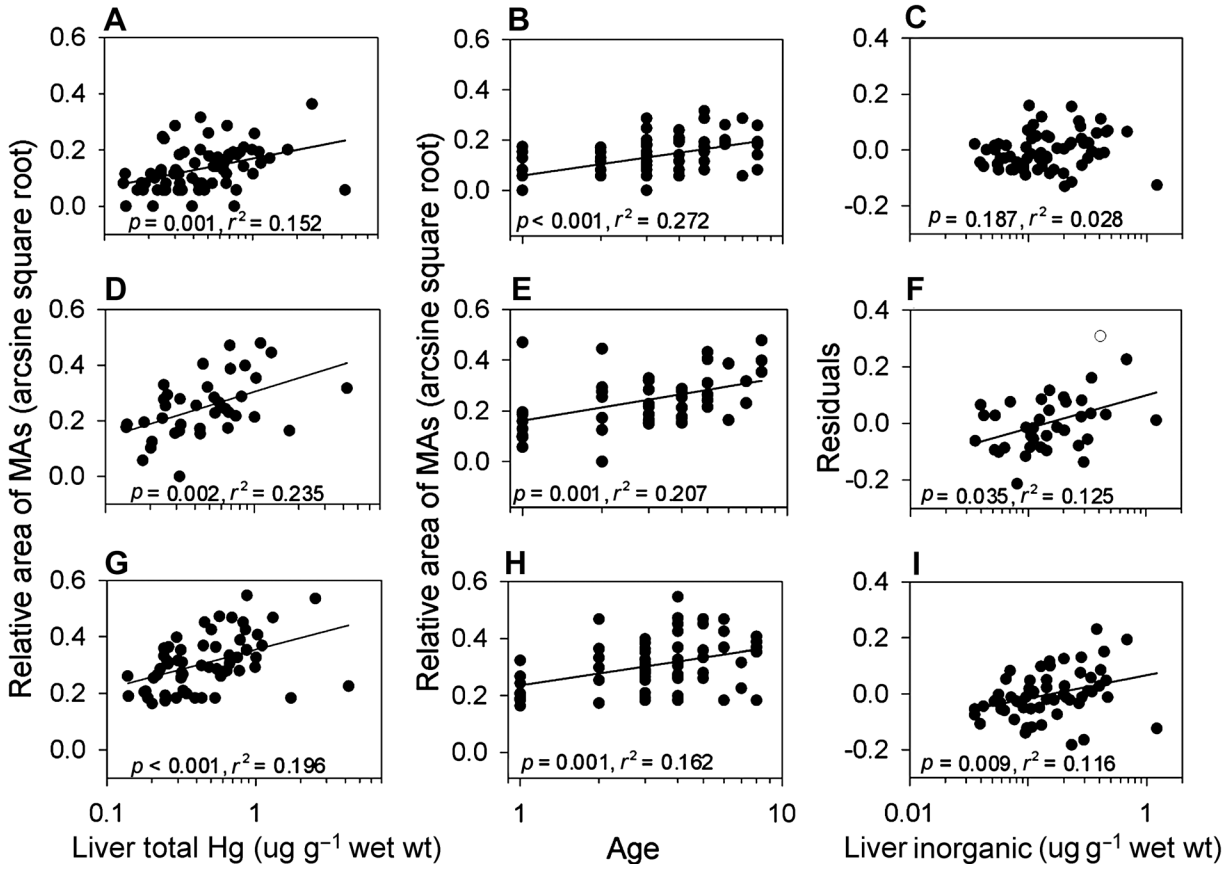

Fig. 3. Linear regressions of the relative tissue area occupied by macrophage aggregates (MAs; arcsine square root transformed) and liver total Hg; of the relative tissue area occupied by MAs and age; and of the residuals of a linear regression between age and the relative tissue area occupied by MAs and liver inorganic Hg concentrations, in liver (**A–C**), kidney (**D–F**), and spleen (**G–I**) tissues from female yellow perch of all lengths. The symbol in white indicates an outlier.

$p = 0.010$, $r^2 = 0.027$), but LSI was unrelated to lake pH. The LSI of females >10 cm was positively related to muscle (THg), but no relationships existed between LSI and tissue Hg concentrations in females ≤10 cm (Table 4).

Condition

The condition of females ranged from 0.75 to 1.45, and differed significantly among lakes ($F_{11,244} = 6.796$, $p < 0.001$; Table 2). These differences in condition, however, were not explained by the differences in muscle or liver Hg concentrations among lakes (Table 2 and Supplemental Data, Table S1). A weak positive relationship existed between the condition of all females and the concentrations of liver THg, liver MeHg, and liver inorganic Hg (Table 4), as well as age ($F_{1,225} = 38.715$, $p < 0.001$, $r^2 = 0.147$), but the condition of these females was unrelated to lake pH. The condition of female perch >10 cm was positively related to the concentration of all three Hg forms in the liver but not to muscle THg, whereas the condition of females ≤ 10 cm was not related to either muscle or liver Hg concentrations (Table 4).

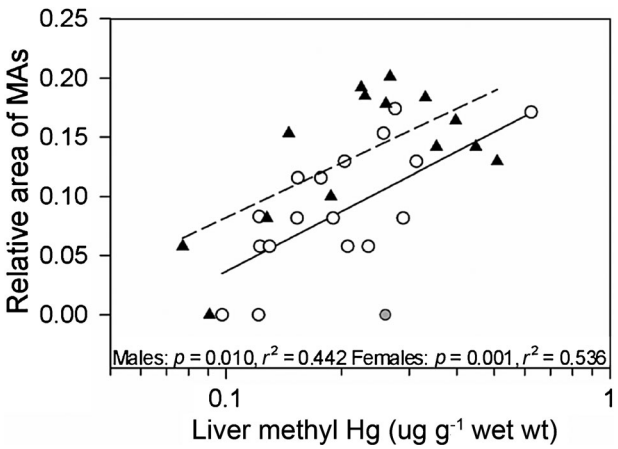

Fig. 4. Linear regression of the relative tissue area occupied by macrophage aggregates (MAs; arcsine square root transformed) and liver methyl Hg concentrations for male (▲) and female (○) yellow perch ≤ 2 years old. Solid and dashed regression lines indicate females and males, respectively.

DISCUSSION

In the present study, the effects of Hg on the health of perch from KNPNHS were assessed by relating perch tissue Hg concentrations to endpoints at the cellular, organ, and whole-body levels (MAs, LSI, and condition, respectively). We found that the prevalence of MAs in tissues, liver size (LSI), and condition were positively related to tissue Hg concentrations. These latter two relationships were unexpected given the negative relationships reported between fish tissue Hg concentrations and LSI [16], and condition (e.g., [9,10]) in fish with lower tissue Hg concentrations than those in perch from KNPNHS. Despite the elevated Hg concentrations in perch from the present study, evidence of adverse health effects due to Hg were only found at the cellular level. The reasons for the absence of effects at the organ and whole-body levels remain

Table 4. Least squares linear regression relationships between the health variables liver somatic index (LSI) and condition (log_{10} transformed) and tissue Hg concentrations ($\mu g\,g^{-1}$ wet wt; log_{10} transformed) in female perch of different groups (≤ 10 cm, > 10 cm, and all lengths)[a]

Perch length	Dependent variable	Tissue Hg species	Slope	Intercept	r^2	p	No.
≤10 cm	Condition	Liver total Hg	0.018	0.018	0.019	0.438	34
		Liver methyl Hg	–0.029	–0.013	0.021	0.412	34
		Liver inorganic Hg	0.015	0.023	0.029	0.338	34
		Muscle total Hg	–0.009	–0.002	0.002	0.709	82
	LSI	Liver total Hg	–0.079	–0.127	0.060	0.168	33
		Liver methyl Hg	–0.072	–0.143	0.022	0.413	33
		Liver inorganic Hg	–0.058	–0.141	0.073	0.128	33
		Muscle total Hg	–0.077	–0.179	0.008	0.431	76
>10 cm	Condition	Liver total Hg	0.034	0.038	0.056	0.004	144
		Liver methyl Hg	0.027	0.045	0.024	0.066	144
		Liver inorganic Hg	0.026	0.047	0.062	0.003	144
		Muscle total Hg	0.021	0.038	0.008	0.232	173
	LSI	Liver total Hg	0.010	–0.105	0.001	0.723	142
		Liver methyl Hg	0.048	–0.084	0.015	0.150	142
		Liver inorganic Hg	–0.015	–0.115	0.004	0.470	142
		Muscle total Hg	0.085	–0.082	0.027	0.034	165
All lengths	Condition	Liver total Hg	0.037	0.037	0.076	<0.001	178
		Liver methyl Hg	0.031	0.044	0.034	0.013	178
		Liver inorganic Hg	0.028	0.045	0.078	<0.001	178
		Muscle total Hg	0.039	0.038	0.034	0.003	255
	LSI	Liver total Hg	–0.010	–0.106	0.001	0.662	176[b]
		Liver methyl Hg	0.020	–0.093	0.003	0.486	176[b]
		Liver inorganic Hg	–0.025	–0.120	0.012	0.148	176[b]
		Muscle total Hg	0.069	–0.092	0.017	0.044	244[c]

[a] $p < 0.05$ was considered statistically significant.
[b] Includes one perch of unknown length.
[c] Includes three perch of unknown lengths.

unclear. However, positive relationships between LSI or condition and tissue Hg concentrations were likely a result of confounding positive relationships with measures of fish size. In these relatively low-productivity lakes, the positive relationships could be an indication of improved condition, liver stores, and Hg associated with increased food consumption.

Tissue mercury concentrations

Studies conducted in the mid-1990s identified the KNPNHS region as a biological hotspot for mercury [18], and the elevated perch muscle Hg concentrations found in the present study support this. For instance, the mean muscle THg concentration of KNPNHS perch ($0.430 \pm 0.014\,\mu g\,g^{-1}$ wet wt) with a mean length of $11.18\,cm \pm 0.19$ exceeds that reported for 20-cm yellow perch across northeastern North America (overall standardized mean $= 0.351 \pm 0.198$ (standard error)) [26]. The range of muscle (THg) (0.08–$2.13\,\mu g\,g^{-1}$ wet wt) for perch from KNPNHS also exceeds the ranges found in yellow perch from other remote locations; more specifically, concentrations ranged from approximately 0.05 to $0.14\,\mu g\,g^{-1}$ (wet wt) in 8-cm standardized yellow perch from remote Canadian shield lakes [27], and from 0.09 to $1.11\,\mu g\,g^{-1}$ (wet wt, means per lake) in 20-cm standardized perch from the Adirondacks [28].

Although the THg concentration in perch muscle was an average of 96.5% MeHg, the percentage of MeHg in liver tissue was lower and far more variable (range: 3.0–85.6%). Similar results have been shown in liver tissue from northern pike (*Esox lucius*; ~28–70%) [14], redear sunfish (*Lepomis microlophus*; 55.8%), spotted gar (*Lepisosteus oculatus*; 2.0%), and largemouth bass (*Micropterus salmoides*; 73.8%) [29]. It is important to understand the accumulation and distribution of both MeHg and inorganic Hg in fish tissues because, based on laboratory studies, the two Hg forms are known to differ in their toxicity to fish [30]. The reason for the lower percentage of MeHg in liver tissue relative to muscle tissue observed across a range of species is unknown, but two likely possibilities exist. The first is in vivo MeHg demethylation, and although this has not yet been proven to occur in fish, this process could result in increased inorganic Hg and lowered percentage of MeHg in the liver. Second, it has been suggested that inorganic Hg ingested by wild fish may be preferentially sequestered in the liver, also resulting in a lower percentage of MeHg [14]. The negative relationship we have shown between percentage of MeHg and THg concentrations in the liver supports either of these hypotheses, as do the data from northern pike [14].

In many perch from KNPNHS, the liver THg concentration exceeded that in the muscle (i.e., liver [THg]: muscle [THg] ratio > 1). This has been reported in other wild freshwater fish [9,14], and inorganic Hg sequestration in liver tissue has been suggested as a cause [14]. Our data showing a negative trend between liver:muscle (THg) ratio and liver percentage of MeHg supports this theory, but the physiological implications of higher liver Hg are unknown.

Macrophage aggregates

The MAs are groups of phagocytic cells that collect the products of tissue breakdown, including cellular debris; accordingly, MA size and numbers increase as a result of increased cellular and tissue degradation [19] due to contaminant exposure, but also to aging [31] or starvation [32]. In the present study, the relative area of tissue occupied by MAs was higher in tissues from fish with higher Hg concentrations, both before (kidney, liver, spleen) and after (kidney and spleen) adjusting for the effects of age. Similar increases in MAs have been reported in the literature. For instance, increases in MAs have been shown in the head kidney of laboratory fish exposed to MeHg [4]. In wild fish, positive relationships existed between the occurrence of liver or kidney MAs and the muscle (THg) of northern pike (~0.21–$0.82\,\mu g\,g^{-1}$ wet wt in muscle) [8], of liver MAs and liver or muscle (THg) of spotted gar

(~0.01–1.1 $\mu g\,g^{-1}$ wet wt in muscle) [24], and of spleen MAs and the (THg) in whole brook trout (~0.03–0.25 $\mu g\,g^{-1}$ wet wt in whole body) independent of age [15]. Our findings suggest that Hg exposure may be causing increased tissue damage in these perch. Furthermore, increases in the occurrence of liver MAs were associated with decreases in liver size, which also suggests a negative impact, although this is in contrast to the positive relationship that existed between the liver size and muscle THg concentrations of these perch in a much larger sample size.

The occurrence of MAs is naturally higher in spleen and kidney tissues than in the liver of fish [33], and our findings agree with this observation. We found the occurrence of MAs in the spleen and kidney of female perch was higher than that in the liver at a given age, or at a given liver inorganic Hg concentration. This lower occurrence of MAs in the liver is likely due to the differing functions of these organs, and may account for the absence of an age-independent relationship in this tissue.

MAs can form in fish tissues due to age, disease, starvation, or nutritional imbalances, or exposure to agents that cause the breakdown of red blood cells (hemolysis) [34]. We found that lake pH was negatively related to the prevalence of MAs in the liver tissue of female perch. Given that lake pH and fish muscle THg concentrations are also negatively related, pH is a potentially confounding factor. To our knowledge, the effects of acidic conditions on MA formation in fish are unstudied, but blood from fish exposed to acidic conditions undergoes hemolysis more readily than unexposed fish [35]. It is therefore possible that the exposure of fish to low pH conditions (such as the lakes within KNPNHS with pH ranging from 4.99 to 6.32) could result in the formation of MAs as a result of increased hemolysis. In the present study, we were unable to distinguish whether the occurrence of liver MAs may be due to the effects of Hg exposure, or to the acidity of KNPNHS lakes, and the effects of pH on MA occurrence should be further investigated.

Previous studies have found the occurrence of tissue MAs to be the same between the sexes of wild yellow perch [31] and brook trout [15]. We found, however, that the relative area occupied by MAs in both the liver and kidney of male perch was higher than that in the females at similar THg concentrations. The MAs are known to be involved in immune function, particularly antigen recognition [19], and the immune response of fish can differ between sexes following contaminant exposure (e.g., [36]). This could explain the sex differences we found; however, the effects of MeHg on fish immune function are not well understood [30].

Given the function of MAs, it can be concluded that increased tissue or cellular damage occurred in perch with higher tissue Hg concentrations. Recently, Hg and MAs were shown to be colocalized in wild fish liver tissue, directly linking Hg to cellular damage [24]. Analyses using LA ICP–MS of perch from the present study support these findings. Although Hg accumulation in perch MAs was generally lower than that observed in spotted gar by Barst et al. [24], 94% of perch MAs contained higher Hg isotope counts than adjacent hepatocytes. This Hg accumulation within liver MAs, in addition to the positive relationships between the prevalence of liver MAs and tissue Hg concentrations, strengthens our hypothesis that Hg exposure results in an increased occurrence of MAs in these perch. Additionally, although Hg and pH cannot be distinguished as possible causes of increased prevalence of MAs, our LA ICP–MS findings provide some evidence toward the former. It is currently unknown whether increases in tissue MAs relate to organ, whole-body, or population level effects in fish.

Liver somatic index

Changes in LSI reflect changes in energy storage and metabolism and can be used to indicate contaminant exposure in fish [37]. The impacts of MeHg exposure on the LSI of laboratory fish, however, appear to be unstudied. We found a positive relationship between perch LSI and muscle (THg), indicating that Hg exposure was not adversely affecting the energy storage of these fish or resulting in other toxic effects detectable at the organ level. This is similar to the positive relationship reported between LSI and ovary (THg) in one walleye (*Sander vitreus*) population (0.25–0.54 $\mu g\,g^{-1}$ wet wt, mean muscle [THg] per site) [38], but in contrast to the negative relationship reported for another (~0.13–3.35 $\mu g\,g^{-1}$ wet wt in liver) [16]. The differences among lakes in female perch LSI in KNPNHS were likely a result of differences among lakes in physical and biological characteristics that may impact LSI, rather than Hg exposure.

Many studies have shown that fish THg concentrations increase with fish length (Wyn, et al. [1]). It is likely, therefore, that the positive relationship between female perch LSI and muscle (THg) resulted from the confounding positive relationship between LSI and a measure of fish size. Liver somatic index is influenced by fish nutritional status [39], and a shift in the diet of larger perch to higher trophic level prey (as suggested for condition below) could result in a concomitant increase in LSI with fish size and muscle (THg).

Condition

Condition of female perch in KNPNHS was positively related to liver and muscle Hg concentrations, and this is in contrast to the negative relationships reported in other studies. For instance, condition was negatively related to whole-body (THg) in yearling yellow perch (~0.03–0.23 $\mu g\,g^{-1}$ wet wt) [40], to muscle (THg) in wild walleye (~0.20–0.38 $\mu g\,g^{-1}$ wet wt) [6], to liver (THg) in northern pike (0.07–0.62 $\mu g\,g^{-1}$ wet wt in muscle) [14], to liver and gonad (THg) in white sturgeon (*Acipenser transmontanus*; 0.020–0.780 $\mu g\,g^{-1}$ wet wt in liver) [10], and to liver, muscle, and blood (THg) in striped bass (*Morone saxatilis*; 0.06–1.06 $\mu g\,g^{-1}$ wet wt in muscle) [9]. The relationship between MeHg and fish condition can be confounded by relationships between condition and fish age in some field studies [41], and a weak positive relationship existed in the present study between perch condition and age. Larger yellow perch consume prey from higher trophic levels (including small fish) [42], and this change in diet could potentially increase their nutritional status and condition, as well as their MeHg intake. A coincident increase in condition and tissue (MeHg) would explain the observed positive relationships.

It is unclear why the condition of perch in the present study was not adversely affected by Hg. Yellow perch are known to be a metal-tolerant species, and are often the only species present in lakes with metal contamination [43]. This tolerance could explain the difference in results between those studies reporting negative relationships with condition and the present study. Female perch condition differed among lakes, but these differences were likely due to variability among the lakes in physical and biological factors that can influence condition (e.g., temperature, water quality, habitat, food availability, and predation), rather than an effect of Hg. Similarly, the acidity of KNPNHS lakes may be an explanation for the lack of evidence of an effect of Hg on condition. Yellow perch are tolerant to low pH [44];

however, their energy allocation toward processes such as growth can be altered in acidic conditions (e.g., Ryan and Harvey [45]). Therefore, it is possible this may be occurring in KNPNHS perch, although we were unable to find a relationship between the condition of females and lake pH.

Within KNPNHS, the THg concentrations that occur in other fish species are comparable to or higher than those in yellow perch. For instance, the mean whole-body THg concentrations ($\mu g\, g^{-1}$ wet wt, assuming 80% moisture content) of other fish species from lakes sampled in the present study range from 0.30 to 1.05 in white perch (*Morone americana*), from 0.23 to 0.44 in white sucker (*Catostomus commersonii*), and from 0.23 to 1.05 in brown bullhead (*Ameiuris nebulosus*; Meredith Clayden, 2011, Master's thesis, University of New Brunswick, Saint John, NB, Canada). If these species are less tolerant to metal contamination than yellow perch, it is possible they may be experiencing adverse health effects due to Hg, a possibility that should be investigated further.

Conclusions and recommendations

Our findings indicate that the health of the yellow perch inhabiting KNPNHS was adversely affected by Hg at the cellular level. In contrast, we found Hg did not adversely affect the higher levels of biological organization within fish, as measured using the endpoints condition and LSI. At the cellular level, increased cellular damage and tissue breakdown were associated with higher tissue Hg in individuals and within the MAs relative to their surrounding hepatocytes, and were higher for males than females at a given Hg concentration. It is currently unknown whether increases in tissue MAs are indicative of impacts on whole-body or population level effects, and why tissues of male perch are more susceptible to adverse effects of Hg than female perch.

The muscle THg concentrations in these perch are lower than those found in other fish species from northeastern North America. Concentrations found in largemouth bass, northern pike, walleye, and lake trout (*Salvelinus namaycush*) are >1.5 times higher than the concentrations found in yellow perch from the same area [26]. Given these concentrations in other fish species, it is possible that the adverse effects we have shown, or further effects, may be occurring in other wild fish populations within northeastern North America. This raises concerns for the health of these populations, and further research is needed to determine the extent of Hg effects on the health of fish populations inhabiting similarly remote ecosystems.

SUPPLEMENTAL DATA

Figs. S1–S2.
Tables S1–S2. (550 KB PDF).

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