

Environmental Toxicology

GENERAL AND HISTOLOGICAL INDICATORS OF HEALTH IN WILD FISHES FROM A BIOLOGICAL MERCURY HOTSPOT IN NORTHEASTERN NORTH AMERICA

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Abstract: Kejimikujik National Park and National Historic Site, Nova Scotia, Canada, is considered a biological mercury (Hg) hotspot because the tissues of yellow perch (*Perca flavescens*) and common loons (*Gavia immer*) inhabiting the lakes frequently exceed so-called safe levels of Hg. In the present study, the relationships between Hg and overall health of males and females of 3 forage fish species (brown bullhead *Ameiurus nebulosus*, banded killifish *Fundulus diaphanus*, and golden shiner *Notemigonus crysoleucas*; $n = 6\text{--}18/\text{sex}/\text{lake}$) in 6 lakes at the park were assessed using condition factor, liversomatic index (LSI), and macrophage aggregates (MAs; indicators of tissue damage). Mean muscle total Hg (THg) concentrations of brown bullhead, banded killifish, and golden shiner across lakes were $0.32\text{ }\mu\text{g/g}$, $0.27\text{ }\mu\text{g/g}$, and $0.34\text{ }\mu\text{g/g}$, respectively. Condition was negatively related to muscle THg in golden shiner and banded killifish, LSI was not related to THg in any species, and all species showed evidence of increasing MA prevalence (counts and area) with increasing THg concentrations. The MAs were most prevalent in spleen tissues of golden shiner, with mean percentage cover ranging from 0.36% to 5.59% across lakes. In addition, the area of MAs appeared to be better predicted by THg concentration than was the number of MAs in the same tissue. These findings suggest that Hg is affecting the health of wild fishes in Kejimikujik National Park and National Historic Site and that other populations with similar or higher concentrations of this metal may also be at risk. *Environ Toxicol Chem* 2016;9999:1–12. © 2016 SETAC

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INTRODUCTION

Mercury (Hg) is a pollutant of global concern, and anthropogenic emissions have increased Hg concentrations in the atmosphere by 2- to 3-fold since the industrial revolution [1]. Mercury has a long residence time in the atmosphere, and even the most remote areas receive Hg from anthropogenic sources through its long-range transport in air currents [2,3]. Once in surface waters, Hg is methylated by bacteria to form methylmercury (MeHg; organic). This form is the greatest concern for aquatic wildlife because it bioaccumulates within organisms and biomagnifies over trophic levels, so that MeHg in fish and fish-eating wildlife can affect their health [4,5].

Methylmercury has numerous effects on the nervous system, as well as on the behaviors, reproduction, and endocrine and immune function of fish. These adverse effects on fish have been well documented in the laboratory, but these studies have provided limited information because some involved less relevant modes of exposure (aqueous rather than dietary) or used exposure concentrations of Hg above environmentally relevant levels. In fish, reproduction appears to be affected by MeHg through inhibition of hypothalamus, pituitary, and gonadal function [6]. For example, in fathead minnows (*Pimephales promelas*) fed diets of $3.93\text{ }\mu\text{g Hg/g}$ dry weight for 250 d, plasma testosterone and 17 β -estradiol levels were suppressed in males and females, respectively, spawning was delayed and decreased spawning success was observed [7].

In addition, estrogen and testosterone levels were negatively correlated with MeHg concentrations in white sturgeon (*Acipenser transmontanus*) from the lower Columbia River (OR, USA) [8]. In some studies (but not all), general health, measured using condition factor and liversomatic index (LSI), has been negatively related to Hg concentrations in wild fish [9,10]. They can frequently exceed the toxicity threshold for total Hg (THg), which is set at $0.2\text{ }\mu\text{g/g}$ wet weight, whole body [11–14]. Even so, few studies have investigated the effects of MeHg on wild fish, and those that exist have mainly focused on larger bodied species.

Macrophage aggregates (MAs) are tissue accumulations of phagocytic white blood cells (macrophages) and 4 pigments (melanin, lipofuscin, ceroid, and hemosiderin) that are found in the kidney, liver, and spleen of teleost fishes. The MAs localize and destroy the products of cell breakdown or cell debris (i.e., red blood cells), as well as foreign materials (i.e., pathogens) [15]. Although MAs occur naturally, increases in the size or number of MAs implies an increased rate of tissue damage and suggest poorer overall health of fish. For these reasons, they have been proposed as histopathological indicators of exposure to degraded environments [16]. Increases in the prevalence of MAs in wild fish from contaminated sites have been consistently observed; livers of the common carp (*Cyprinus carpio*) and barbel (*Barbus barbus*) from polychlorinated biphenyl- and polybrominated diphenyl ether-contaminated sites, respectively, had higher numbers of, size, and percentage area covered by MAs compared with fish from reference sites [17,18]. The incidences of MAs also appear to be correlated with exposure to Hg. The percentage area occupied by spleen MAs in brook trout (*Salvelinus fontinalis*) across the western United States and liver MAs in spotted gar (*Lepisosteus oculatus*) from

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Caddo Lake, Texas (USA) were positively related to their muscle THg concentrations [12,19].

At Kejimikujik National Park and National Historic Site in Nova Scotia, Canada, a known biological Hg hotspot, the yellow perch (*Perca flavescens*) and common loons (*Gavia immer*) inhabiting the lakes have concentrations well above the threshold for toxicity. The Hg levels in fish and fish-eating wildlife are high at Kejimikujik National Park and National Historic Site because of elevated MeHg at the base of the food web [20]. Even though these fish frequently exceed this safety threshold, previous studies on yellow perch have found that condition factor and LSI are not negatively related to their tissue Hg levels, nor is reproductive health being affected, as measured by gonadosomatic index and germ cell development [13,21]. However, MAs in kidney, liver, and spleen tissues were greatest in fish with the highest Hg concentrations [13,22]. Other fish species inhabiting the lakes occupy a similar trophic position to that of perch, overlap in their concentrations of THg, and therefore also exceed the threshold for Hg toxicity (B. Wyn, 2007, Master's thesis, University of New Brunswick, Saint John, NB, Canada). Nothing is known about the health of these other species and whether there is any correlation of these endpoints with tissue Hg.

The objective of the present study was to examine whether the health of forage fishes at Kejimikujik National Park and National Historic Site was negatively related to their MeHg concentrations (measured as THg in muscle; ~95% MeHg [23]). We investigated whether the incidence and area of MAs in 3 different tissues were related to muscle THg concentrations in 3 forage fishes from these lakes. The general health of these fishes was also assessed through examination of relationships between condition factor or LSI and tissue THg concentrations.

MATERIALS AND METHODS

Study site and sample collection

Located in southern Nova Scotia, Canada, Kejimikujik National Park and National Historic Site is an area removed from large industrial centers, and is mainly used for tourism and recreational activities [24]. High Hg concentrations in biota from this park are the result of long-range atmospheric transport and deposition, inputs from the watersheds, and the characteristics of the lakes that promote Hg methylation and bioaccumulation, such as low pH and high organic carbon content in lake waters [24]. The area has been the focus of several previous studies, and the results of 1 such study on Hg in yellow perch was used to choose lakes that represent a gradient of mercury exposure, with fish concentrations ranging from below the threshold of toxicity to concentrations well above this threshold (B. Wyn, 2007, Master's thesis, University of New Brunswick, Saint John, NB, Canada). The lakes and their respective THg concentrations (mean THg $\mu\text{g/g} \pm$ standard deviation wet wt) in yellow perch were: Upper Silver ($0.14 \pm 0.06 \mu\text{g/g}$), Big Dam East ($0.17 \pm 0.08 \mu\text{g/g}$), Cobrielle ($0.21 \pm 0.08 \mu\text{g/g}$), Hilchemakaar ($0.23 \pm 0.10 \mu\text{g/g}$), Puzzle ($0.28 \pm 0.11 \mu\text{g/g}$), and North Cranberry ($0.37 \pm 0.11 \mu\text{g/g}$). In each of the 6 lakes, 10 male and 10 female brown bullhead (*Ameiurus nebulosus*), banded killifish (*Fundulus diaphanus*), and golden shiner (*Notemigonus crysoleucas*) were targeted for collection. Fish were collected in September 2013 or October 2014 using fyke nets (final sample sizes ranged from 6 to 18 for

males and females in each lake). Fork length (or total length for banded killifish; ± 1 mm) and total weight (± 0.1 g) were recorded. Fish were euthanized via spinal severance according to procedures approved by the University of New Brunswick Animal Care Committee (Canada), the liver was weighed (± 0.001 g), gonads were collected to sex fish, and liver, kidney (anterior and posterior separated; kidney could not be collected for banded killifish), and spleen were collected for histological analysis. A sample of dorsal muscle above the lateral line (skin removed) was taken from each fish for THg (used as a proxy for MeHg; 95% MeHg in fish muscle [23]) analysis. Weight and length measurements were used to calculate condition factor ($100 \times [\text{total weight } \{\text{g}\} / \text{fork length}^3 \{\text{cm}\}]$) and LSI was calculated using liver weight and total weight ($100 \times [\text{liver weight } \{\text{g}\} / \text{total weight } \{\text{g}\}]$).

Tissue mercury analysis

Muscle samples were weighed into glass cryovials, and then lyophilized using a Labconco Freezone 12. The THg concentrations were measured using a Milestone DMA-80 Direct Mercury Analyzer. Wet weight concentrations were calculated using the percentage moistures of individual fish. All mercury data are presented as wet weight concentrations unless otherwise stated. Quality assurance and quality control measures (QA/QC) included method blanks, sample duplicates, and standard reference materials (SRM), and these were run after every set of 10 samples. Duplicate relative percentage difference (mean \pm standard error [SE]) of every 10th sample was $12.8 \pm 5.4\%$ ($n = 40$). The mean percentage recovery of the SRM DORM-4 (fish protein certified reference material, National Research Council of Canada) was $91.8 \pm 0.64\%$ ($n = 47$). The percentage recovery for the intralaboratory standard was $97.6 \pm 2.23\%$ ($n = 55$), and the percentage recovery for the 10-ng liquid mercury standard was $98.7 \pm 1.7\%$ ($n = 56$). The mean concentration of method blanks was $0.0018 \mu\text{g/g}$ dry weight ± 0.0004 ($n = 58$). The limit of detection, calculated as the sum of 3 times the standard deviation of method blanks and the mean concentration of method blanks, was $0.0102 \mu\text{g/g}$ dry weight. All measures passed QA/QC guidelines.

Tissue methylmercury analysis

Three banded killifish and 3 brown bullhead muscle samples representing a range of THg concentrations were selected to determine the percentage of THg present as MeHg. The percentage of MeHg for golden shiner was determined in a previous study at Kejimikujik National Park and National Historic Site (M.G. Clayden, 2011, Master's thesis, University of New Brunswick, Saint John, NB, Canada). The MeHg was measured using a Brooks Rand Model II system at Acadia University (Wolfville, NS, Canada), using methods similar to those of Edmonds et al. [25]. The MeHg was measured in muscle tissue following digestion in 1 mL of 25% KOH/MeOH solution [26]. Samples were analyzed through ethylation with NaB (C_2H_3)₄, purge and trap gas chromatography, and detection was done using atomic fluorescence spectrometry. Each sample batch included method and analytical blanks, method and analytical replication, and an SRM (DOLT-4). Sample masses ranged from 9.999 mg to 10.665 mg dry weight. Mean (\pm SE) recovery for SRMs were $94.5 \pm 1.7\%$ ($n = 6$). The concentrations of MeHg in method blanks ($n = 6$) averaged 2.43 ng/g and were below the instrument detection limits, which were set at 3 times the standard deviation of the method blanks.

Macrophage aggregate analysis

To prepare liver, anterior kidney, and spleen for histological analysis, tissues were dehydrated with ethanol and embedded in paraffin wax. Tissues were cut into 5- μm -thick sections, and a total of 3 sections were mounted on glass microscope slides for each sample. Samples were randomly assigned to staining batches and stained with hematoxylin and eosin.

Macrophage aggregates (Figure 1) were quantified using methods similar to those of Barst et al. [14]. One slide (3 sections) for each fish tissue was examined. Using a Leica 2500 DM light microscope mounted with a Leica DFC290 camera, 3 representative pictures of each tissue section were taken at 100 \times magnification, for a total of 9 pictures per sample. These pictures were imported into ImageJ software, where the

area of each MA was measured. Macrophage aggregates are easily visualized under the microscope because of the pigments, and MAs were identified as dark brown clusters of cells that contrasted with the pink- or orange-stained cytoplasm and contents, and the blue-stained nuclei of the cells (Figure 1). The average area of MAs and the average number of MAs in the field of view were calculated for the 9 pictures. The percentage cover of MAs (calculated by dividing the sum of the area of all MAs in a field of view by the total area of the field of view [$1.44 \times 10^6 \mu\text{m}^2$] and multiplying by 100) was calculated to make direct comparisons with previous studies.

Statistical analyses

All statistics were performed using the MASS, lme4, glmmADMB, or base packages in R (R Core Team 2013). The factors affecting THg, condition, LSI, MA number, and MA area were determined using generalized linear mixed models (GLMMs). These were used because errors in the dependent variables were not normally distributed, and the most appropriate models included both fixed and random variables. The combinations of independent variables that best explained the variability in THg, condition, LSI, MA number, and MA area were determined. Then THg, condition, LSI, and MA area were assessed using gamma distribution with an inverse link function, and MA number was assessed using negative binomial distribution with a log link function. For THg, the fixed factors were length and sex. For condition, the fixed factors were THg and sex. For LSI, MA number, and MA area, the fixed factors were THg, length, and sex. Models with condition factor or LSI as the dependent variable, and MA number or MA area as the independent variable, were also done to determine whether MAs may be negatively affecting fish health. Lake was treated as a random factor in all models because it was not of primary interest, and the lakes used in the present study were chosen at random from a larger set of available lakes. Random effects assessed included different intercepts among lakes (shown as 1|lake), different intercepts and slopes of the dependent variable versus THg among lakes (shown as 1+THg|lake), different intercepts and slopes of the dependent variable versus length among lakes (shown as 1+length|lake), and different intercepts and slopes of the dependent variable versus length and versus THg among lakes (shown as 1+THg+length|lake).

The Akaike Information Criterion (AIC) method was used to determine what variables should be included in the model. For each dependent variable, the model with the lowest AIC value was chosen as the best model. The best combination of random and fixed factors was compared with null (meaning no fixed factors) GLM and GLMMs to determine whether the best combination of variables was better than an intercept-only model. Pseudo R^2 values were calculated to determine how much variation was explained by the model. A function written specifically for calculating pseudo R^2 values of GLMMs in R was used to determine how well each model explained the variability in the dependent variable [27]. This function calculated the pseudo R^2 for the entire model (random and fixed effects). The pseudo R^2 was also calculated for the null model (no fixed effects and random intercepts among lakes), and this value was subtracted from the R^2 for the full model, giving a partial R^2 for just the fixed effects. When a GLM (model with no random effect) was found to be the best model, a pseudo R^2 was calculated using the equation $([\text{Null deviance} - \text{Residual deviance}]/\text{Null deviance})$.

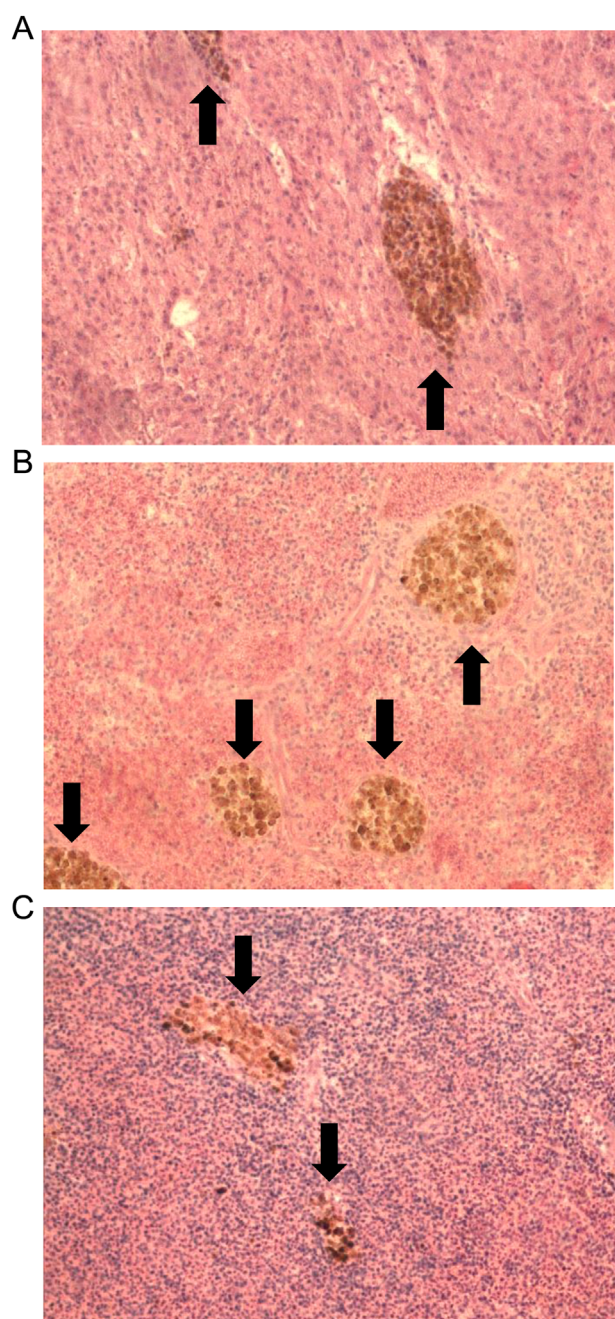


Figure 1. Macrophage aggregates (indicated with black arrows) in brown bullhead liver (A), banded killifish spleen (B), and golden shiner spleen (C) tissue sections, photographed at 100 \times magnification.

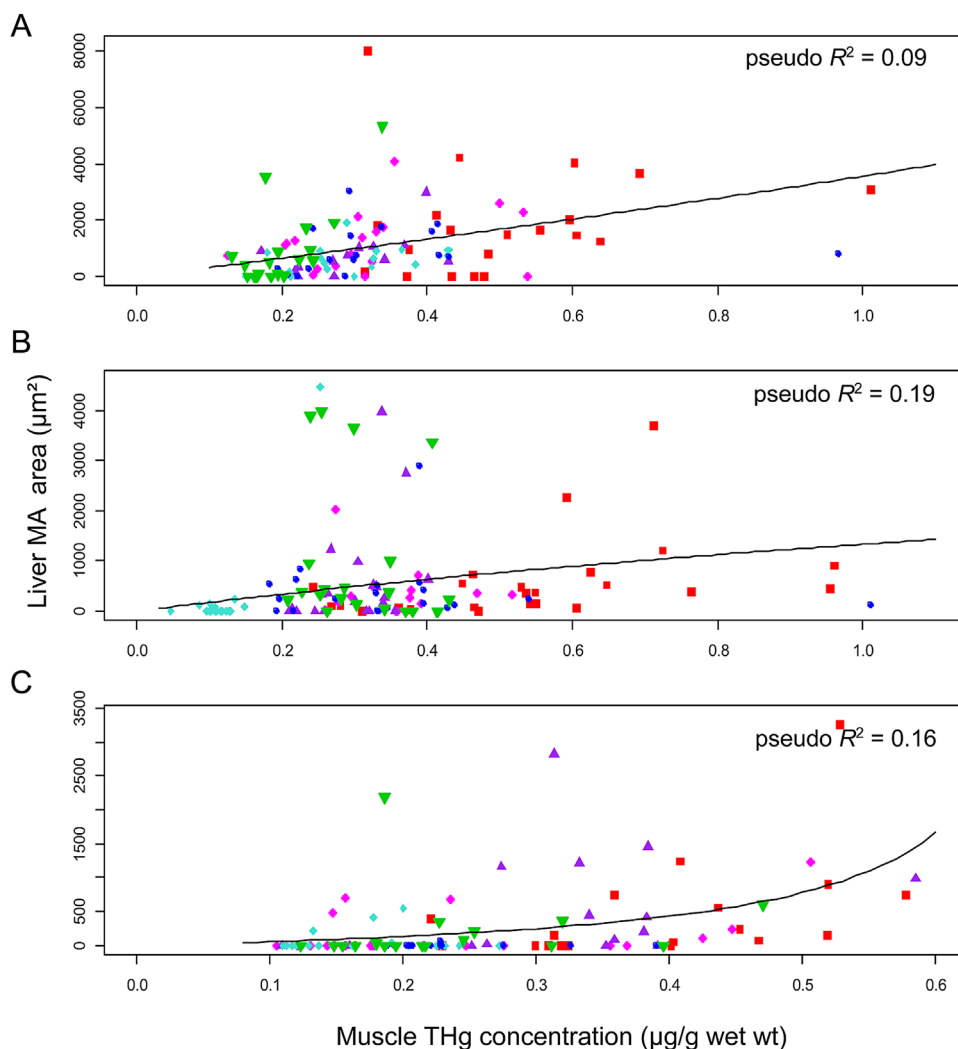


Figure 2. Relationships between liver macrophage aggregate (MA) area and muscle total Hg (THg) concentration ($\mu\text{g/g}$ wet wt) of brown bullhead males and females (A; sexes combined), golden shiner males and females (B; sexes combined), and banded killifish males and females (C; sexes combined) across all lakes (pseudo R^2 shown). Colors and symbols represent individuals from different lakes with increasing mean Hg concentration (turquoise diamonds: Upper Silver, blue circles: Big Dam East, pink circles: Hilchemakaar, purple triangles: Cobrielle, green triangles: Puzzle, red squares: North Cranberry). Black line represents line of fit for overall relationship of all lakes combined.

In addition, differences in MA number or MA area within species among tissues were assessed with tissue as a fixed factor (random factor 1lake). Using the slope estimates from these models, the tissues were ranked from highest to lowest MA area or number. Finally, within-tissue, among-species models included species as a fixed factor (random factor 1lake) and only fish within a similar range of Hg concentrations ($0.11\text{--}0.58\text{ }\mu\text{g/g}$ wet wt). The line of fit for data that were plotted (see Figures 2, 3, and 4) was calculated as $(XV/\text{slope} + [\text{intercept} \times XV])$ where XV is a vector of x values (THg concentrations) within the observed range of THg concentrations, slope is the slope estimate for the fixed effect of THg concentration, and intercept is the y-intercept estimate of the model. For models that included more than 1 fixed factor, only the effect of THg is shown in the figures.

RESULTS

Tissue mercury concentrations

Muscle THg concentrations of brown bullhead males and females ranged from $0.13\text{ }\mu\text{g/g}$ to $1.01\text{ }\mu\text{g/g}$ wet weight across lakes, and the mean concentration in the highest THg lake

(North Cranberry, $0.50\text{ }\mu\text{g/g}$) was 2.5 times higher than the lowest THg lake (Puzzle, $0.20\text{ }\mu\text{g/g}$). Muscle THg concentrations of golden shiner ranged from $0.05\text{ }\mu\text{g/g}$ to $1.01\text{ }\mu\text{g/g}$ wet weight, and fish in North Cranberry (mean = $0.55\text{ }\mu\text{g/g}$) had 4.6 times higher THg than those in Upper Silver Lake (mean = $0.12\text{ }\mu\text{g/g}$). For banded killifish, THg concentrations ranged from $0.11\text{ }\mu\text{g/g}$ to $0.58\text{ }\mu\text{g/g}$ wet weight, with fish from North Cranberry (mean = $0.39\text{ }\mu\text{g/g}$) having 2.3 times more Hg than Upper Silver (mean = $0.17\text{ }\mu\text{g/g}$). The percentage of THg that was MeHg in banded killifish averaged $82.03 \pm 8.78\%$ ($n = 3$), and for brown bullhead it averaged $90.86 \pm 7.55\%$ ($n = 3$).

Liver tissue macrophage aggregates

The area of MAs in the liver tissue of brown bullhead males and females ranged from $0\text{ }\mu\text{m}^2$ to $8010\text{ }\mu\text{m}^2$ across lakes (Table 1). The best model for predicting MA area in liver tissue of brown bullhead across lakes included just THg concentration with a positive slope, with no random effect (GLM; Table 2 and Figure 2). The number of MAs in the liver tissue of brown bullhead males and females ranged from 0 to 23 across all lakes. The best model for predicting MA number included THg

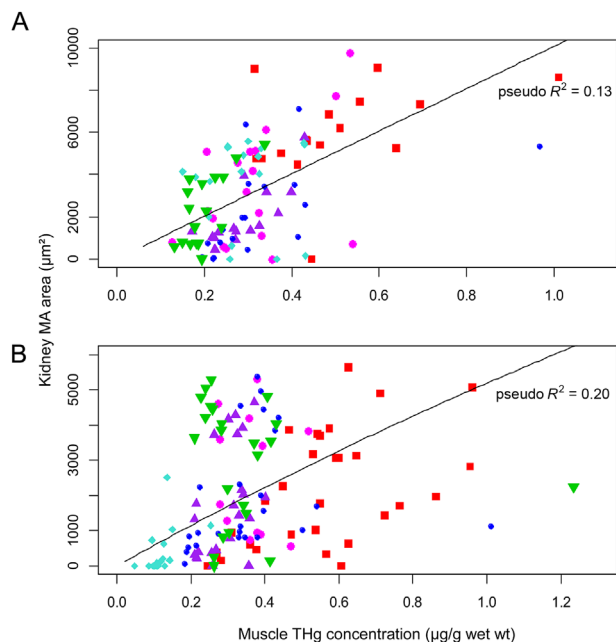


Figure 3. Relationships between kidney macrophage aggregate (MA) area and muscle total Hg (THg) concentration ($\mu\text{g/g}$ wet wt) of brown bullhead males and females (A; sexes combined) and golden shiner males and females (B; sexes combined) across all lakes (pseudo R^2 shown). Colors and symbols represent individuals from different lakes with increasing mean Hg concentration (turquoise diamonds: Upper Silver, blue circles: Big Dam East, pink circles: Hilchemakaar, purple triangles: Cobrielle, green triangles: Puzzle, red squares: North Cranberry). Black line represents line of fit for overall relationship of all lakes combined.

(positive slope), length (positive slope), and sex, with no random effect (GLM; Table 3). The MA number in liver tissue of females was higher than that of males (Table 3). The MA number was better predicted by the fixed factors than MA area, and THg, length, and sex were all significant in predicting the number of MAs in liver of brown bullhead.

The area of MAs in liver tissue of golden shiner males and females ranged from $0 \mu\text{m}^2$ to $4472 \mu\text{m}^2$ across lakes. The THg and length both predicted liver MA area in golden shiner males and females across lakes with no random effect (GLM; Table 2 and Figure 2). The number of MAs in liver tissue of golden shiner ranged from 0 to 24 across lakes. The THg and length both predicted liver MA number, with different intercepts among lakes (random effect 1lake, GLMM; Table 3). The MA area was better predicted by the fixed factors than MA number, and the fixed factors THg and length were significant in predicting the area of MAs in liver of golden shiner across lakes.

The area of MAs in liver tissues of banded killifish males and females ranged across lakes from $0 \mu\text{m}^2$ to $3248 \mu\text{m}^2$. The best model for predicting liver MA area in banded killifish across lakes included THg, with no random effect (GLM; Table 2 and Figure 2). The number of MAs in liver tissue of killifish males and females ranged from 0 to 14 across lakes. The MA number was predicted by length, with different intercepts among lakes (random effect 1lake, GLMM; Table 3). The MA number was better predicted by the fixed factors than MA area, and length was best at predicting the number of MAs in liver of banded killifish.

Spleen tissue macrophage aggregates

The area of MAs in spleen tissues of brown bullhead males and females ranged across lakes from $0 \mu\text{m}^2$ to $9350 \mu\text{m}^2$. The best model for predicting spleen MA area in brown bullhead

across lakes included THg, with no random effect (GLM; Table 2). The number of MAs ranged from 0 to 18 in males and females and was best predicted by THg, with different intercepts among lakes (random effect 1lake, GLMM; Table 3). The MA number and MA area were predicted equally as well by the fixed factors, and THg concentration was best at predicting both the area and the number of MAs in spleen tissue of brown bullhead.

The area of MAs in spleen tissues of male and female golden shiner varied across lakes from $0 \mu\text{m}^2$ to $7263 \mu\text{m}^2$. The MA area was best predicted by THg, with no random effect (GLM; Table 2). Spleen tissue MA number in golden shiner ranged from 0 to 55 and was best predicted by length, with different intercepts among lakes (random effect 1lake, GLMM; Table 3). The MA number was better predicted by the fixed factors than MA area, and length was best at predicting the number of MAs in spleen of golden shiner.

The area of MAs in spleen tissues of banded killifish males and females ranged across lakes from $0 \mu\text{m}^2$ to $10130 \mu\text{m}^2$. The best model for predicting MA area included THg with no random effect (GLM; Table 2). The MA number in spleen tissue of banded killifish ranged from 0 to 44, and the best model included length with different intercepts among lakes (random effect 1lake, GLMM; Table 3). The MA number and MA area were predicted equally as well by the fixed factors, but length best predicted MA number, whereas THg best predicted MA area in spleen tissue of banded killifish.

Kidney tissue macrophage aggregates

The area of MAs in kidney tissues of brown bullhead males and females ranged from $0 \mu\text{m}^2$ to $9792 \mu\text{m}^2$ across lakes. The best model for predicting kidney MA area included THg but no random effect (GLM; Table 2 and Figure 3). The MA number in kidney tissues of brown bullhead ranged across lakes from 0 to 27, and the best model included both THg and length, with different intercepts among lakes (random effect 1lake, GLMM; Table 3). The MA area was better predicted by the fixed factors than MA number, and THg was best at predicting the area of MAs in kidney tissue of brown bullhead.

The area of MAs in kidney tissues of golden shiner males and females ranged from $0 \mu\text{m}^2$ to $5638 \mu\text{m}^2$ across lakes. The best model for predicting kidney MA area in golden shiner across lakes included both THg and length, with no random effect (GLM; Table 2 and Figure 3). The MA number ranged from 0 to 19 in males and females across lakes and was also best predicted by THg and length, with significantly different intercepts among lakes (random effect 1lake, GLMM; Table 3). The MA area was better predicted by the fixed factors than was MA number, and THg and length were significant in predicting the area of MAs in kidney tissue of golden shiner.

Differences in macrophage aggregate area and number among tissues

For both sexes of brown bullhead combined, MA area was predicted by tissue and there was no random effect (GLM). Across all lakes, mean MA area was highest for kidney tissue (mean area = $3228 \mu\text{m}^2$), followed by spleen ($2312 \mu\text{m}^2$), and then liver ($1072 \mu\text{m}^2$). The number of MAs was also predicted by tissue, and there was no random effect (GLM). Across all lakes, the mean number of MAs was slightly higher in spleen (mean number = 5.4) than kidney (5.3) and was lowest in the liver (2.7).

For golden shiner, with males and females combined, MA area was predicted by tissue and there was no random effect (GLM). Across all lakes, mean MA area was highest in the

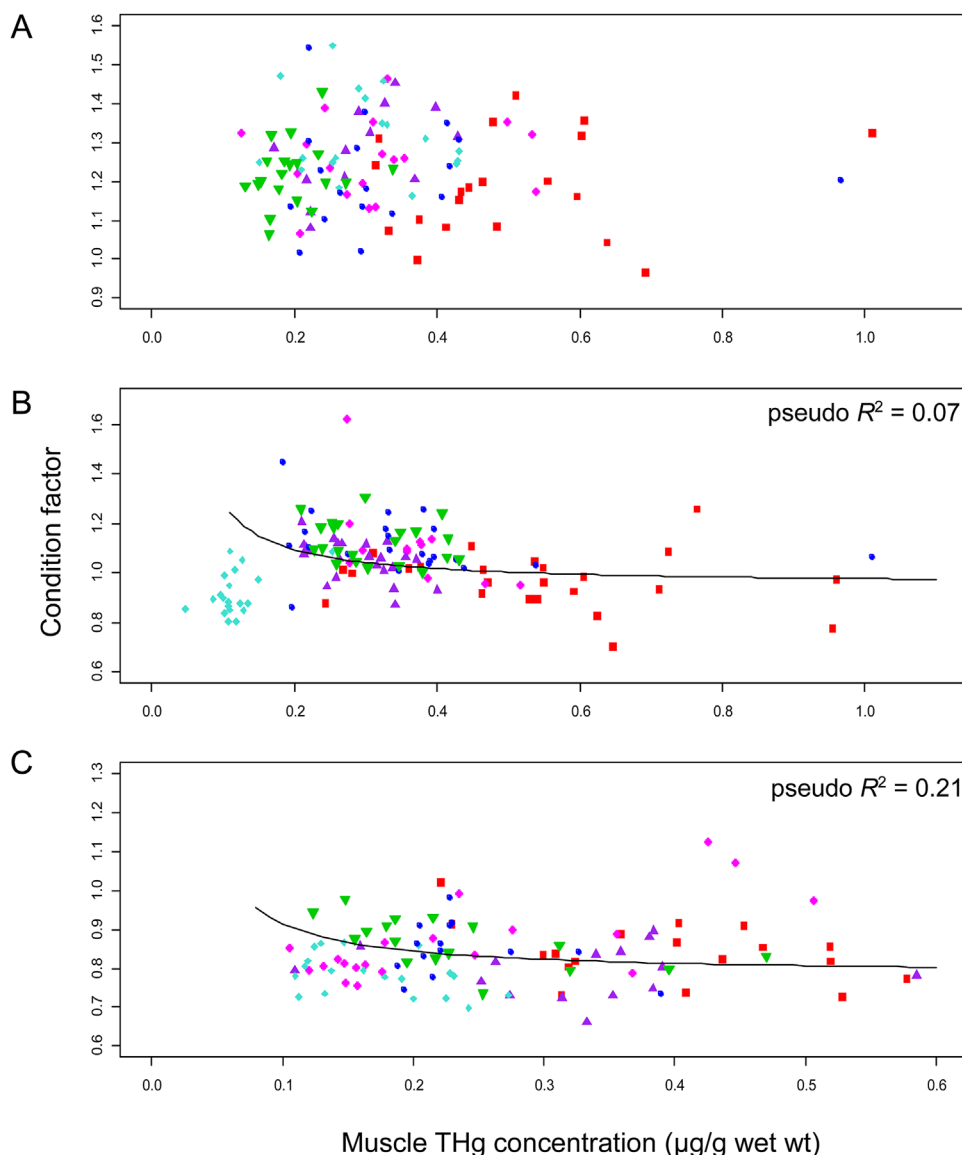


Figure 4. Relationships between condition factor and muscle total Hg (THg) concentration ($\mu\text{g/g}$ wet wt) of brown bullhead males and females (A; sexes combined), golden shiner males and females (B; sexes combined), and banded killifish males and females (C; sexes combined) across all lakes (pseudo R^2 shown). Colors and symbols represent individuals from different lakes with increasing mean Hg concentration (turquoise diamonds: Upper Silver, blue circles: Big Dam East, pink circles: Hilchemakaar, purple triangles: Cobrielle, green triangles: Puzzle, red squares: North Cranberry). Black line represents line of fit for overall relationship for all lakes combined.

spleen ($2680 \mu\text{m}^2$), followed by kidney ($1927 \mu\text{m}^2$), and then liver ($517 \mu\text{m}^2$). Mean number of MAs was also predicted by tissue, and there were different intercepts among lakes (random effect 1lake, GLMM). The number of tissue MAs was highest in the spleen (17.8) followed by kidney (5.1) and then liver (1.8) for golden shiner.

For all banded killifish combined, MA area was predicted by tissue and there was no random effect (GLM). Across all lakes, mean MA area was higher in the spleen ($2105 \mu\text{m}^2$) than the liver ($252 \mu\text{m}^2$). The number of MAs was also predicted by tissue, and there were different intercepts among lakes (random effect 1lake, GLMM). The number of tissue MAs was higher in the spleen (4.8) than liver (0.4) for banded killifish.

Differences in macrophage aggregate area and number among species

Within the range of THg concentrations that overlapped among all 3 species (0.11–0.58 $\mu\text{g/g}$ wet wt), MA area in spleen tissue was not different among species but there were different

intercepts among lakes (random effect 1lake, GLMM). However, the number of MAs in the spleen was predicted by species (fixed factor), and there were different intercepts among lakes (random effect 1lake, GLMM). The number of MAs in spleen tissue was highest in golden shiner across all lakes, followed by brown bullhead, and then banded killifish.

The MA area in liver tissue was predicted by species (fixed factor) with no random effect; mean liver MA area was largest for brown bullhead, followed by golden shiner and then banded killifish (GLM). The mean number of MAs was also predicted by species (fixed factor), with significantly different intercepts among lakes (random effect 1lake, GLMM). Brown bullhead had the highest mean number of MAs in liver tissue across all lakes, followed by golden shiner and then killifish.

The MA area in kidney tissue was predicted by species (fixed factor), with no random effect (GLM). Mean kidney MA area was higher for brown bullhead than golden shiner. The mean number of MAs in kidney tissue was not predicted by species (GLMM).

Table 1. Summary data (mean \pm standard deviation) for male and female brown bullhead, golden shiner, and banded killifish from Kejimikujik National Park, Nova Scotia

Species	Sex	Length (cm)	Weight (g)	Condition factor	LSI (%)	Muscle THg ($\mu\text{g/g}$ wet wt)	Tissue	MA cover (%)	MA area (μm^2)	MA (no.)
Brown bullhead	Male	15.3 \pm 2.1	47.5 \pm 21.0	1.24 \pm 0.11	1.77 \pm 0.46	0.30 \pm 0.13	Spleen	1.160 \pm 1.684	2042 \pm 1726	5.8 \pm 4.9
							Liver	0.273 \pm 0.391	938 \pm 1255	2.0 \pm 2.1
							Kidney	0.882 \pm 1.139	3186 \pm 2389	5.1 \pm 3.9
	Female	15.4 \pm 2.1	49.7 \pm 22.6	1.27 \pm 0.13	2.29 \pm 1.02	0.35 \pm 0.12	Spleen	1.128 \pm 1.456	2567 \pm 2025	5.1 \pm 3.4
							Liver	0.580 \pm 1.128	1198 \pm 1280	3.4 \pm 4.3
							Kidney	1.069 \pm 1.213	3270 \pm 2536	5.4 \pm 5.0
Golden shiner	Male	8.9 \pm 2.0	8.7 \pm 5.8	1.04 \pm 0.14	1.26 \pm 0.41	0.31 \pm 0.15	Spleen	3.514 \pm 4.297	2666 \pm 2083	18.0 \pm 9.6
							Liver	0.258 \pm 0.994	415 \pm 740	1.6 \pm 3.4
							Kidney	0.455 \pm 0.577	1912 \pm 1746	4.8 \pm 4.2
	Female	9.0 \pm 2.0	8.9 \pm 5.8	1.04 \pm 0.13	1.35 \pm 1.15	0.37 \pm 0.19	Spleen	3.313 \pm 3.009	2282 \pm 1813	17.5 \pm 9.4
							Liver	0.259 \pm 0.724	621 \pm 1138	2.1 \pm 3.2
							Kidney	0.529 \pm 0.658	1940 \pm 1549	5.1 \pm 4.1
Banded killifish	Male	7.7 \pm 1.0	4.0 \pm 2.4	0.83 \pm 0.08	1.68 \pm 0.53	0.26 \pm 0.12	Spleen	1.43 \pm 1.67	1933 \pm 1860	5.0 \pm 7.2
							Liver	0.028 \pm 0.059	235 \pm 576	0.5 \pm 1.9
	Female	8.0 \pm 1.3	4.5 \pm 1.9	0.82 \pm 0.07	1.75 \pm 0.62	0.28 \pm 0.12	Spleen	1.39 \pm 1.397	2307 \pm 1929	4.3 \pm 6.4
							Liver	0.059 \pm 0.133	271 \pm 532	0.5 \pm 1.1

LSI = liversomatic index; THg = total mercury; MA = macrophage aggregates.

Table 2. Results of generalized linear models for predicting macrophage aggregate area in the liver, spleen, and kidney of brown bullhead, golden shiner, and banded killifish by the fixed factors total mercury, sex, and length of fish from Kejimikujik National Park and National Historic Site, Nova Scotia^a

Tissue	Species	Slope THg (\pm SE)	Slope length (\pm SE)	Intercept (\pm SE)	pR ²
Liver	Brown bullhead	$3.18 \times 10^{-4} \pm 8.22 \times 10^{-5}$	0.06 \pm 0.02	$-3.95 \times 10^{-5} \pm 2.23 \times 10^{-4}$	0.09
	Golden shiner	$5.49 \times 10^{-4} \pm 2.32 \times 10^{-4}$		$-5.60 \times 10^{-3} \pm 1.65 \times 10^{-3}$	0.19
	Banded killifish	$2.03 \times 10^{-3} \pm 6.96 \times 10^{-4}$		$-2.79 \times 10^{-3} \pm 1.77 \times 10^{-3}$	0.16
Spleen	Brown bullhead	$7.64 \times 10^{-5} \pm 2.53 \times 10^{-5}$		$1.78 \times 10^{-4} \pm 8.27 \times 10^{-5}$	0.04
	Golden shiner	$6.46 \times 10^{-5} \pm 1.97 \times 10^{-5}$		$1.81 \times 10^{-4} \pm 6.64 \times 10^{-5}$	0.09
	Banded killifish	$1.35 \times 10^{-4} \pm 2.74 \times 10^{-5}$		$-2.56 \times 10^{-5} \pm 9.13 \times 10^{-5}$	0.16
Kidney	Brown bullhead	$9.86 \times 10^{-5} \pm 1.59 \times 10^{-5}$	$6.32 \times 10^{-3} \pm 1.90 \times 10^{-3}$	$-6.86 \times 10^{-7} \pm 4.52 \times 10^{-5}$	0.13
	Golden shiner	$1.64 \times 10^{-4} \pm 2.71 \times 10^{-5}$		$-6.60 \times 10^{-4} \pm 2.01 \times 10^{-4}$	0.20

^aResults for sex are not shown because they were never included in the best model. Shown are intercepts and slopes (\pm SE) of significant fixed effects, and pR² values for the best models. Empty spaces in the slope columns indicate that the variable was not in the best model. No random effect of lake was observed. SE = standard error; THg = total mercury; pR² = pseudo R².

Table 3. Results of generalized linear models for predicting macrophage aggregates number in the liver, spleen, and kidney of brown bullhead, golden shiner, and banded killifish by the fixed factors total mercury, sex, and length of fish from Kejimikujik National Park and National Historic Site, Nova Scotia^a

Tissue	Species	Slope THg	Slope length	Slope sex	Intercept	Random effect	pR ² (fixed)
Liver	Brown bullhead	1.80 \pm 0.35	0.16 \pm 0.03	-0.35 \pm 0.12	-1.72 \pm 0.46		0.47
	Golden shiner	1.27 \pm 0.58	0.23 \pm 0.06		-1.61 \pm 0.59	1lake	0.11
	Banded killifish		0.33 \pm 0.03		-2.30 \pm 0.56	1lake	0.18
Spleen	Brown bullhead	0.94 \pm 0.41			1.51 \pm 0.16	1lake	0.04
	Golden shiner		0.16 \pm 0.03		1.47 \pm 0.25	1lake	0.11
	Banded killifish		0.20 \pm 0.06		0.05 \pm 0.53	1lake	0.16
Kidney	Brown bullhead	1.23 \pm 0.47	0.07 \pm 0.03		0.26 \pm 0.44	1lake	0.07
	Golden shiner	1.08 \pm 0.38	0.25 \pm 0.04		-0.94 \pm 0.37	1lake	0.09

^aShown are intercepts and slopes (\pm standard error) of significant fixed effects, random effects, and pR² values for the fixed effects only for the best models. Empty spaces in the slope or random effect columns indicate that the variable was not in the best model. THg = total mercury; pR² = pseudo R².

Condition factor

The condition factor of brown bullhead males and females ranged from 0.97 to 1.64 across all lakes and both sexes. Condition factor of bullhead males and females varied among lakes (random effect 1lake), although the variability was not explained by THg concentration or sex (GLMM; Table 4 and Figure 4). For golden shiner the condition factor ranged from 0.56 to 1.62 across lakes and both sexes. Condition factor

was inversely related to THg concentrations of golden shiner across lakes; it was not related to sex, and there were differences in the slope of this relationship among lakes (random effect 1+THglake, GLMM; Table 4 and Figure 4). Golden shiner from Upper Silver were significantly smaller than fish from other lakes, which resulted in significantly lower condition factors, but including these fish in the analysis did not appear to change the result. Banded killifish condition factor ranged from 0.66 to 1.12 across lakes and for both sexes. Condition factor

Table 4. Results of generalized linear mixed models for predicting condition factor and liversomatic index by the fixed factors total mercury, sex, and length of brown bullhead, golden shiner, and banded killifish from Kejimikujik National Park and National Historic Site, Nova Scotia, with lake as a random factor^a

Species	Dependent variable	Slope sex	Slope THg	Intercept	Random effect	pR ² (full)	pR ² (fixed)
Brown bullhead	Condition			0.80 ± 0.02	1lake	0.14	
Golden shiner	Condition		−0.04 ± 0.02	1.05 ± 0.05	1+THglake	0.43	0.07
Banded killifish	Condition		−0.02 ± 0.02	1.28 ± 0.09	1+THglake	0.39	0.21
Brown bullhead	LSI	0.11 ± 0.02		0.48 ± 0.05	1lake	0.54	0.14
Golden shiner	LSI			0.82 ± 0.06	1lake	0.25	
Banded killifish	LSI			0.60 ± 0.05	1lake	0.23	

^aResults for length are not shown because they were never included in the best model. Shown are intercepts and slopes (± standard error) of fixed effects, random effects, and pR² values of the full model (full) and fixed effects only (fixed) for the best models. Empty spaces in the slope columns indicate that the variable was not in the best model.

THg = total mercury; pR² = pseudo R².

was not predicted by sex but was inversely related to THg, and the slope of this relationship differed among lakes (random effect 1+THglake, GLMM; Table 4 and Figure 4).

LSI

The LSI of brown bullhead ranged from 0.63 to 2.75 for males and from 1.12 to 3.16 for females across all lakes. Sex was the best predictor of LSI for brown bullhead, with males having lower LSI than females, and different intercepts among lakes (random effect 1lake, GLMM; Table 4). The LSI of all golden shiners ranged from 0.39 to 3.69 (Table 4) and was not predicted by THg, sex, and length, but intercepts differed among lakes (random effect 1lake, GLMM; Table 4). The LSI of banded killifish ranged from 0.24 to 3.85 across all lakes and for both sexes (Table 4). As for golden shiner, THg, sex, and length were not predictive of LSI in killifish, but intercepts differed among lakes (random effect 1lake, GLMM; Table 4).

DISCUSSION

In the present study, THg and health endpoints at the cellular, organ, and whole-body level were compared in 3 species of fish collected from a biological Hg hotspot to understand the relationship between overall health of wild fishes and their Hg concentrations. With few exceptions, MA area and number in kidney, liver, and spleen tissues were positively related to muscle THg concentrations in brown bullhead, banded killifish, and golden shiner, and condition factor was negatively related to THg in banded killifish and golden shiner, but no exposure-related changes in LSI were observed. The area of MAs appeared to be better predicted by Hg concentrations than was the number of MAs in the same tissue, suggesting that the increasing size of MAs may be a specific response to contaminant exposure. Differences in MA area or number among species and tissues were found, but for the most part, no differences between sexes were observed. The present study provides evidence that overall size and pathologies in tissues were related to THg accumulation in forage fishes.

Tissue mercury concentrations

Muscle THg concentrations in the fish species collected for the present study were lower when compared with those recently measured in yellow perch at Kejimikujik National Park and National Historic Site [13], but still support the classification of this area as a biological Hg hotspot. Across lakes, mean THg concentrations (± SE) of brown bullhead (0.32 ± 0.01 µg/g) and golden shiner (0.34 ± 0.01 µg/g) were similar, and were closer to that of yellow perch (0.43 ± 0.01 µg/g) than banded killifish (0.27 ± 0.01 µg/g). These forage fish feed

primarily on invertebrates at maturity, whereas yellow perch are known to shift food sources with age, becoming piscivorous as adults [27]; their higher trophic position as adults would increase their Hg accumulation. In addition, yellow perch also have a longer lifespan (9–10 yr [28]) than the other species, especially compared with banded killifish, which have a lifespan of approximately 4 yr [29], and perch can therefore accumulate more Hg in their tissues over time than the shorter lived species.

The percentages of THg that consisted of MeHg in muscle tissue varied among forage fish species and lakes but were typically lower than those of previous studies. Banded killifish and brown bullhead mean percentage of MeHg in muscle (82.03% and 90.86%, respectively) measured in the present study were similar to that of golden shiner from these lakes (86.21 ± 8.51% in North Cranberry and 93.98 ± 4.09% in Puzzle; B. Wyn, 2007, Master's thesis, University of New Brunswick, Saint John, NB, Canada), but these were lower than the >95% MeHg previously reported for 7 freshwater and 8 saltwater fish species [23]. The freshwater species were larger bodied fish such as largemouth bass (*Micropterus salmoides*), yellow perch, northern pike (*Esox lucius*), and white sucker (*Catostomus commersoni*), and their percentage of MeHg in muscle tissue was more comparable to that of yellow perch from Kejimikujik National Park and National Historic Site (95.17 ± 2.54% in North Cranberry and 99.05 ± 17.54% in Puzzle; B. Wyn, 2007, Master's thesis, University of New Brunswick, Saint John, NB, Canada). The proportion of MeHg is known to increase with increasing trophic position, because MeHg is more efficiently transferred from prey to predator, with top predators having almost 100% MeHg in their tissues [30–32]. Although the concentrations are variable, it is evident that most Hg in fish muscle tissue is MeHg, and MeHg is likely the primary Hg species causing adverse effects in wild fish at the Kejimikujik National Park and National Historic Site.

Macrophage aggregates

In the present study, the area of MAs was positively related to THg concentrations in 3 species and in 3 different tissues, and the number of MAs was positively related to THg concentrations in 2 of the 3 species, suggesting that Hg exposure is linked to MA development in wild fish. The results also support the use of MAs as an indicator of Hg exposure. This has been consistently found in multiple studies, including 2 previous ones at Kejimikujik National Park and National Historic Site; the relative area occupied by MAs in the liver, kidney, and spleen tissue of yellow perch was positively related to their muscle THg concentration across lakes [13,22]. In brook trout, the percentage area occupied by spleen MAs and whole-body THg

concentrations were positively related across 14 lakes in western US National Parks [12]. For spotted gar, the relative areas occupied by liver MAs and muscle THg concentrations were positively related [19]. Similarly, in northern pike the number of MAs in the liver, spleen, and kidney were positively related to muscle THg concentrations [33]. Collectively, these results suggest that increased MAs are indicative of increased Hg exposure, but a follow-up laboratory study should be done to confirm that environmentally relevant Hg concentrations can result in higher incidences or sizes of tissue MAs. One such study has been done on zebrafish (*Danio rerio*) exposed to MeHg through feed spiked with 0.12 μg or 4.11 μg Hg/g dry wt for 78 d. Only liver tissue was analyzed for MAs, and none were found in this tissue (B.D. Barst, 2010, Master's thesis, University of North Texas, Denton, TX, USA). It is possible that the exposure time was not long enough to induce the formation of MAs. Also, zebrafish is a more advanced teleost, in which MAs tend to form primarily in spleen and kidney tissues, not in the liver.

Percentage cover of MAs in the liver of the 3 species of forage fish examined in the present study was similar to the previously studied yellow perch in the Kejimikujik National Park and National Historic Site. Brown bullhead appeared to have higher mean MA percentage cover in the liver compared with yellow perch (0.46% and 0.33%, respectively), with Hg concentrations that were within the range of brown bullhead Hg concentrations [22]. Mean MA percentage cover in the liver of golden shiner (0.25%) and banded killifish (0.042%) across all lakes was lower than that of bullhead and perch. In comparison with yelloweye rockfish (*Sebastes ruberrimus*) in Alaska, with a mean muscle THg concentration of $0.52 \pm 0.30 \mu\text{g/g}$ wet wt, liver MA cover was higher (1.1% in rockfish) than in fish at Kejimikujik National Park and National Historic Site, and mean spleen MA cover was much higher in rockfish (6.6%) compared with brown bullhead (0.96%), golden shiner (1.6%), and banded killifish (0.99%) [14]. Higher MA cover would be expected given the higher mean Hg concentration for rockfish, and they are a much longer lived species (collected fish were ~5–120 yr old) [14]. However, comparisons of the number or area of MAs between studies should be made with caution because seasonal changes have been observed in some species. For example, MAs in spleen tissues are highest and lowest in barbel (*Barbus peloponnesius*) collected in the fall and summer, respectively [34]. Similarly, MAs in kidney tissues of ohrid trout (*Salmo letnica*) increase from pre to late vitellogenesis and then decrease after spawning [35]. If MAs are to be used as an indicator of exposure to MeHg, consistent sampling methods will need to be implemented to account for factors like seasonal variability, and, based on the results from previous work, fall could be the most appropriate sampling season.

In the 3 fish species we examined, liver tissue had a smaller number of MAs, and the MAs were smaller in area compared with those in kidney or spleen tissue, although there appeared to be no difference in the amount of variability explained by the models among the 3 tissues. The MAs in liver tissue of northern pike were also described as being smaller in size and frequency compared with those of kidney and spleen [33]. Lower MA numbers and area in the liver support the current understanding, because they are more common in tissues where blood is made [36]. In more derived fishes, such as the ones we studied, kidney is the primary hematopoietic tissue. It is unknown why spleen and kidney MA numbers were very similar in brown bullhead, or why spleen MA number was higher than that of the liver and kidney tissue in golden shiner. It may be that the

specific region of the kidney that was dissected was too far posterior, where there are more nephrons and the function shifts toward osmotic regulation rather than hematopoiesis [37], or high splenic MA production could simply be characteristic of the species. Based on the results of the present study, kidney may be the most reliable tissue as an indicator for Hg exposure because both number and size of MAs were related to THg in both species, with high R^2 values relative to the other tissues; however, the kidney was also the most difficult of the 3 organs to sample.

The THg did not appear to predict MA area and MA counts equally well. When THg was in the model, predictability was higher for MA area than for MA number (see pR^2 values in tables), suggesting a stronger relationship between MA area and THg concentrations. This trend was also seen in previously studied yellow perch: THg was a stronger predictor of MA area than MA number [22; A.K. Muller, 2015, Master's thesis, Aachen University, Aachen, Germany]. In all models, Hg was a significant predictor of MA area, but in 3 of the 8 models MA number was not related to Hg concentration. The MAs are known to increase with a variety of factors such as age, starvation, and disease. It is possible that the increasing size of MAs is an adaptive response to higher metal exposure, whereas the increased formation of individual MAs is a response to other factors. Area of MAs also seemed to be less sensitive in terms of fixed and random effects. In 6 of 8 models, the best model included just THg as the fixed factor. In all models, there was no random effect of lake. For MA number, only 1 of 8 models included just THg as the fixed effect, and only 1 of 8 models had no random effect. The lack of differences in MA area among lakes could be more evidence that the size of MAs is a response to metals, whereas increasing the number of MAs is a response to other factors that could vary among lakes, such as food availability. Interestingly, hepatic lysosomes in yellow perch also increased in size in individuals with higher Hg concentrations [22]. Increasing the size of MAs and lysosomes, which are both heavily involved in detoxification processes, may be an adaptive response to Hg exposure.

In contrast, fish length was more predictive of the number of MAs than MA area in tissues. Length was a significant factor in 7 of 8 models with MA number as the dependent variable, but was only significant in 2 of 8 models with MA area as the dependent variable. This might suggest that as fish get older, more MAs are formed, rather than the existing MAs getting larger. There was no significant relationship between MA area and length or MA number and length in yellow perch from these same lakes, but these fish only ranged in length from 10 cm to 12 cm [22]. In male pike collected from the Oder River, Germany, the number of MAs in kidney tissue was correlated to fish length, but no significant relationships were found between MA area and fish length [33]. Few studies, with the exception of those mentioned previously, have compared predictors of MA number with those of MA area. Further work could be done to determine broader trends for these 2 measures across species, and to examine whether number of MAs is best predicted by length whereas area of MAs is best predicted by Hg.

The variability explained by the models in the present study ranged from 4% to 45%, demonstrating that MAs in brown bullhead, golden shiner, and banded killifish from Kejimikujik National Park and National Historic Site were likely influenced by a variety of factors, not just Hg. In fish, MAs are known to increase with age, starvation, infection, as well as physical or chemical factors such as low dissolved oxygen [38–41]. They can also differ between sexes, seasons, and stages of

maturation [34,35]. Although we included sex and length (as a proxy for age) in our models and kept the timing of sampling consistent among lakes, it is possible that water quality or food availability were also contributing to the presence of MAs in these species.

Species differences in MA number were evident in the present study on Kejimikujik National Park and National Historic Site lakes, indicating either possible differences in species sensitivity to Hg or inherent differences in the prevalence of MAs among species. Golden shiner had the highest incidences of MAs in spleen tissue compared with those of brown bullhead and banded killifish, and brown bullhead had the highest incidences in liver tissue compared with those of golden shiner and banded killifish at a given THg concentration. There was no difference in the amount of MAs in kidney tissue of golden shiner and brown bullhead. Golden shiner showed the highest number of MAs of all 3 species, but MA number in the spleen was not predicted by THg for this species. Therefore, the differences in MA numbers among species may be because of among-species variability or non-Hg factors, rather than different sensitivities to Hg. Species differences in MA area were evident as well, with brown bullhead having the largest MAs in kidney and liver tissue. There was no difference in MA area in spleen tissue among the 3 species. Size (and age) could be contributing to some of the among-species differences. Brown bullhead generally had the largest (and the most) MAs, and they are the longest lived species, reaching a maximum age of 9 yr. Golden shiner are smaller and reach a slightly shorter maximum age of 8 yr, and banded killifish are the smallest of the 3 species studied, reaching a maximum age of 4 yr [29]. Although age seems to be related to species differences in MAs, aging was not done in the present study. The size of MAs may also be an indication of adaptation because brown bullhead had the largest liver and kidney MAs, and they are very well known for being a tolerant species [28]. Area of MAs was strongly related to THg concentration and could mean that larger MAs are an adaptation to increased Hg exposure. With respect to the threshold for toxic effects (0.33 $\mu\text{g/g}$ wet wt muscle; converted from 0.2 $\mu\text{g/g}$ whole body wet wt [10] by B. Wyn, 2007, Master's thesis, University of New Brunswick, Saint John, NB, Canada), 31% of banded killifish, 38% of brown bullhead, and 50% of golden shiner exceeded the threshold of toxicity; however, differences in the percentage of each species exceeding this threshold did not appear to explain species differences in the number or area of MAs.

Although an increase in MAs is considered to be an indicator of greater tissue damage, whether this increase affects the overall health of fish is unknown. The MAs may replace hepatocytes in the liver or hematopoietic tissue in the kidney and spleen and could impact the other functions of these tissues. This means there is less tissue for other processes, such as metabolism, glycogen or lipid storage, and detoxification in the liver, and immune responses in the kidney and spleen [42]. In the present study condition factor increased with increasing number of MAs in killifish liver and golden shiner spleen tissues, and condition factor increased with golden shiner liver MA area, in contrast to the negative relationship between MAs and condition factor found by Schwindt et al. [12]. At Kejimikujik National Park and National Historic Site, LSI was only negatively related to MA area of golden shiner kidney and liver tissues. The MAs in perch at Kejimikujik National Park and National Historic Site, as well as yelloweye rockfish from Alaska, had elevated Hg within them compared with the

surrounding tissue, indicating that MAs may be an important site of Hg storage and detoxification in fish [13,14,22].

A lack of negative relationships between condition factor or LSI and MAs does not necessarily mean that MAs do not affect overall health of fish. There is evidence that MAs impair the functioning of adjacent cells, further suggesting that other cell and tissue processes would be inhibited [43]. The MAs are associated with acid phosphatase activity and can lead to the increased production of reactive oxygen species and subsequent damage to surrounding cells [37]. Ultrastructural analysis of liver tissues from Kejimikujik National Park and National Historic Site yellow perch showed some increased sizes and heterogeneity of lysosomes and fewer extracellular phagocytes in higher Hg fish (with higher MA areas), but the hepatocytes showed no pathological alterations [22]. Within MAs, melanin is involved in the oxidative stress response by neutralizing free radicals and other toxic substances, lipofuscin is known as a so-called wear and tear pigment and increases with age as tissues degrade, and hemosiderin is most closely linked to the degradation of red blood cells [38]. Although we did not stain and identify specific pigments within the MAs in the present study, correlating the presence of specific pigments to Hg concentrations may give further insight into the mechanisms underlying MA production and their role(s) in detoxification.

Condition factor and LSI

Average condition factor of fish varied among lakes, and the condition factor of golden shiner and banded killifish (but not brown bullhead) was inversely related to their muscle THg concentrations across lakes. Many factors (such as age, season, stage of maturation, food quality, and food availability) influence fish condition [44,45]. There is also evidence that contaminant exposure, including Hg, can affect fish condition [46,47]. In previous studies, condition factor was negatively related to liver THg concentration of northern pike at Isle Royale and gonad and liver Hg concentrations in white sturgeon from the lower Columbia River [8,9]. The negative relationship indicates that at higher concentrations, Hg may negatively affect energy storage of fish. This could be a result of fish expending more energy for processes like metal detoxification [48]. However, this relationship could be confounded by the effect of length (or age). Calculating Fulton's condition factor assumes isometric growth, but fish growth is allometric and therefore this measure of condition can decrease with increasing length [49]. In addition, recent analysis showed that slow-growing northern pike from the Laurentian Great Lakes accumulated more Hg than fast-growing fish in the same lakes suggesting that fish condition drives mercury concentrations rather than vice versa [50]. Nonetheless, the observed relationship may be a concern for forage fish and higher trophic level predatory fish at Kejimikujik National Park and National Historic Site, as well as for fish in other areas with similar levels of Hg. No known laboratory studies have confirmed the inverse relationship between condition factor and Hg exposure at concentrations typically seen in wild fish.

Despite the above considerations, the lack of relationship observed in the present study between Hg and condition factor for brown bullhead is not uncommon. For example, largemouth bass condition was not different between high (mean THg 5.42 $\mu\text{g/g}$ wet wt) and low (mean THg 0.3 $\mu\text{g/g}$ wet wt) Hg lakes in New Jersey [10]. Also, yellow perch previously collected at Kejimikujik National Park and National Historic Site showed among-lake differences in condition that were not related to muscle THg concentrations, and there was a significantly

positive correlation between THg concentration and condition factor of perch of all lengths (K.L. Batchelar, 2011, Master's thesis, University of New Brunswick, Saint John, NB, Canada).

The LSI was not related to THg concentrations of brown bullhead, golden shiner, or banded killifish, suggesting that there were no effects on energy storage in the liver at these Hg concentrations. The LSI is largely influenced by nutritional status, but it can also decrease in fish exposed to contaminants, mainly because of an increase in stress [51,52]. Both organic and inorganic Hg cause oxidative stress, and can lead to lipid peroxidation and decreased lipid reserves in the liver, which could decrease overall liver size and amount of energy stored [9,53]. The relationship between LSI and contaminants is variable because LSI can also increase in response to organic contaminants as a result of hypertrophy [49]. A negative relationship between LSI and THg has been observed in largemouth bass from 3 lakes in New Jersey [10] and walleye from several lakes in the province of Quebec [54]. Of the 3 endpoints (condition factor, LSI, and MAs) examined in the present study, LSI was the least related to Hg exposure.

The present study was done in a national park with no development and related sources of contaminants in the watersheds that could explain the among-lake patterns in fish endpoints. However, there may be other factors that have contributed to these trends such as differences in water quality among systems that are driven by local bedrock geology, as has been observed for wetlands in this park [55]. Several elements (Cd, Cr, Fe, Mg, Ni, Se, and Zn) were detected in muscle tissue of yellow perch collected from 5 of the same lakes in 2013, but none showed the same patterns as those of Hg, and element concentrations were generally similar across systems although sample sizes were low (K.A. Kidd, unpublished data; $n = 2\text{--}5/\text{lake}$). However, the low aqueous pH of lakes, which ranged from 6.32 in Upper Silver to 5.50 in North Cranberry, may also be a contributing factor to the observed decreases in fish health. Acidified water is a known stressor for fish, with sublethal effects such as elevated secretion of the stress hormone plasma cortisol and avoidance behavior seen at pH 6 or below [56,57]. Low-pH water can negatively affect immune and reproductive systems, and the increased stress may lead to decreased feeding activity and, subsequently, condition factor of fish [56]. However, low pH is 1 factor that promotes Hg methylation and MeHg transfer through food webs; therefore the present study is not able to differentiate between the effects of Hg and low pH on fish health. A more extensive field study, or a laboratory study, is needed to determine which factors can affect MAs, LSI, or condition.

CONCLUSIONS

The present study demonstrated that Hg was negatively related to the overall health indicator condition factor (but not in all fish species), and was positively related to a proposed cellular level indicator of Hg exposure (macrophage aggregates) across multiple tissues and species. The models suggest that THg is a better predictor of MA area than MA number, and that MA number may be more affected by non-Hg factors such as fish age. Overall, these results suggest that Hg concentrations in forage fish at Kejimikujik National Park and National Historic Site are high enough to affect the health of some fish and that Hg concentrations in these lakes are of concern. It is also important to note that if concentrations of Hg are high enough in forage fishes to cause concern, then predatory fish and fish-eating wildlife are at even greater risk for toxic effects given the

biomagnification of Hg in the food web. Presently, relatively little is known about MAs and whether they directly affect the overall health of fish. Gaining knowledge about MAs will aid in determining if, and how, they can be used as indicators of Hg exposure and/or effects. The MAs are promising as an indicator because they have consistently been shown to be positively related to contaminant concentrations in several species in the wild. It is important to continue examining how increased MAs may adversely affect overall health of fish, and for this reason, focused laboratory studies are recommended to assess the mechanisms underlying MA formation and subsequent higher level effects in fish exposed to Hg. Finally, Hg concentrations in forage fish at Kejimikujik National Park and National Historic Site overlap with concentrations in several fish populations in similar ecosystems across North America, suggesting that other populations are at similar risk for Hg toxicity, and future research should focus on examining the health of other wild fish populations.

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Data Availability—Data are available on request by contacting Stephanie Graves at University of Saskatchewan (s.graves@usask.ca).

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