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Dietary and Waterborne Mercury Accumulation by Yellow Perch: A Field Experiment

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ABSTRACT: It is well accepted that the majority of monomethylmercury (MMHg) in fish originates in their food; however, the additional contribution of water as a source to fish MMHg levels remains unclear. We used isotope enriched mercury (Hg) in a controlled field experiment to quantify the uptake of Hg from ingested and aqueous sources by young-of-year yellow perch (*Perca flavescens*). Water and zooplankton from a lake that had received ^{202}Hg-enriched additions (called spike Hg) for 7 y during a whole-ecosystem loading study (METAALICUS) provided natural, low-level Hg exposure. We achieved separation of exposure pathways by housing perch in one of four treatments: clean water + clean food; clean water + Hg spiked food; Hg spiked water + clean food; Hg spiked water + Hg spiked food. Fish accumulated MMHg directly from water, and this source accounted for at least 10% of MMHg in fish during the 27-d trial. Accumulation of spike Hg from water and food was additive, with food providing the majority of spike MMHg taken in by fish. Predictions from a bioenergetics model that excludes water as a source underestimated Hg in perch by 11%. This study illustrates the importance of acknowledging both food and water as sources of Hg to fish and suggests that aqueous Hg should be included as a source of contamination in bioaccumulation models and experiments.

INTRODUCTION

Mercury (Hg) is a neurotoxin for many animals[1] including humans[2] and is a common cause of fish consumption advisories in North America.[3] The organic form of Hg, monomethylmercury (MMHg), biomagnifies in aquatic food webs.[1] Organisms at the highest trophic levels such as game fish, which are the mainstay of subsistence, commercial, and recreational fisheries, typically exhibit the highest concentrations of MMHg.[4] Because human exposure to MMHg is primarily through the consumption of contaminated fish, much research has focused on the sources and exposure pathways of fish, in addition to factors influencing the accumulation of this toxin.[5] The relative contributions of dietary and waterborne Hg (inorganic mercury, Hg^{2+}, and MMHg) to MMHg concentrations in fish remains an area of diverse opinion. Initial attempts to quantify and model MMHg accumulation by fish showed that fish MMHg concentrations result from cumulative uptake from food and water.[6−8] In the mid-1990s, however, accumulation studies began to disregard water as a source of MMHg to fish, seemingly prompted by research showing that food is the dominant exposure pathway.[9] Water as a source of MMHg to fish is now ignored in many models and laboratory and field-based studies (e.g., refs 10 and 11). Although fish derive less MMHg from water than food, the extent to which fish accumulate this toxin from water, and seasonal fluctuations in accumulation patterns, remain key questions to resolve in order to better understand and predict fish MMHg concentrations.[12]

Studies that have quantified relative importance of food and water as sources of Hg to fish have yielded variable results. To our knowledge, only two studies have attempted to address this question under natural conditions, but both clearly demonstrate accumulation of Hg through respiration, suggesting that small-bodied fish can derive up to 38% of their Hg directly from water.[9,13] Laboratory estimates of the contribution of Hg uptake from the dissolved phase range from negligible[14] to 10−50% of the total Hg in fish.[15,16] Mercury bioaccumulation models have also yielded variable estimates, ranging from <0.1%[17] to 10%.[18] It is important to note that some Hg accumulation models include water as a source of MMHg to fish (e.g., 18), while others do not (e.g., 19).

Previous field studies have faced two key design issues: exchange of Hg between live prey and water, and among-individual

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variability in initial Hg concentrations.[9] The use of live prey (e.g., zooplankton) as a food source more closely mimics fish dietary preferences, but live organisms actively feed, respire, and excrete wastes such that water and zooplankton Hg concentrations quickly come into equilibrium with each other, preventing complete separation of food and water Hg exposure pathways.[9,13] A further challenge has been the inability to distinguish Hg accumulated during the study from that initially present in fish (t_0). Typically, Hg concentrations in individual fish at the conclusion of a study are compared to an initial Hg concentration averaged for all fish. Many fish species show wide individual variation in Hg concentrations, even among similarly sized individuals,[20] and this "average" approach inherently increases the variability in estimates of Hg accumulation and complicates interpretation of Hg accumulation data.

The use of Hg that is enriched in one of its stable isotopes is an emerging tool that is providing new insights into the fate of Hg in aquatic ecosystems.[21,22] Enrichment is achieved through a variety of atomic engineering methods, including photochemical, electromagnetic, and centrifugal separation.[23] Isotope enriched Hg behaves like ambient Hg, but can be distinguished analytically from indigenous (ambient) Hg.[24] As such, it is possible to apply isotope enriched Hg to an ecosystem and subsequently track its fate. For example, Hg enriched in stable isotopes ^{202}Hg, ^{200}Hg, and ^{198}Hg has been used to examine Hg accumulation in aquatic food webs in several field studies (e.g., 21, 25, 26).

We conducted a replicated field experiment to estimate the relative contributions of dietary and waterborne Hg to young-of-year (YOY) yellow perch (*Perca flavescens*) in a seminatural setting. The ability to distinguish isotope enriched Hg (termed "spike Hg") from ambient Hg provided a unique opportunity to quantify the relative contributions of Hg in food and water to fish. The design of this experiment allowed us to overcome many challenges faced by previous laboratory and field studies through (i) reliable separation of the two Hg exposure pathways and (ii) baseline YOY yellow perch spike Hg concentrations of zero. In addition, we predicted fish Hg accumulation with a bioenergetics-based model that assumes food is the only route of Hg exposure to fish.[19] The objectives of this study were (i) to quantify Hg accumulation from aqueous and dietary pathways by YOY yellow perch, and (ii) to observe the ability of a bioenergetics-based Hg accumulation model to predict the uptake of Hg by YOY yellow perch. Overall, this study aims to discern whether water should be considered an important source of mercury to fish.

EXPERIMENTAL SECTION

Study Location. This experiment took place at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada (49° 39′ 14″ N, 93° 43′ 18″ W) (Figure 1). Lake 658 is an 8.4 ha, circumneutral, headwater lake in the Precambrian Shield that has received additions of isotope enriched Hg as part of a whole-ecosystem loading study (Mercury Experiment To Assess Atmospheric Loading in Canada and the United States, METAALICUS). From 2001 to 2007, inorganic Hg (Hg^{2+}) enriched in the stable isotope ^{202}Hg (to 90.8%; as $HgCl_2$) was mixed into the surface waters of Lake 658 by boat every two weeks during the open water season.[27] $^{202}Hg^{2+}$ (spike Hg^{2+}) was added to achieve an annual loading of 22 $\mu g \cdot m^{-2} \cdot yr^{-1}$, which is approximately 6-fold higher than local ambient Hg deposition.[26]

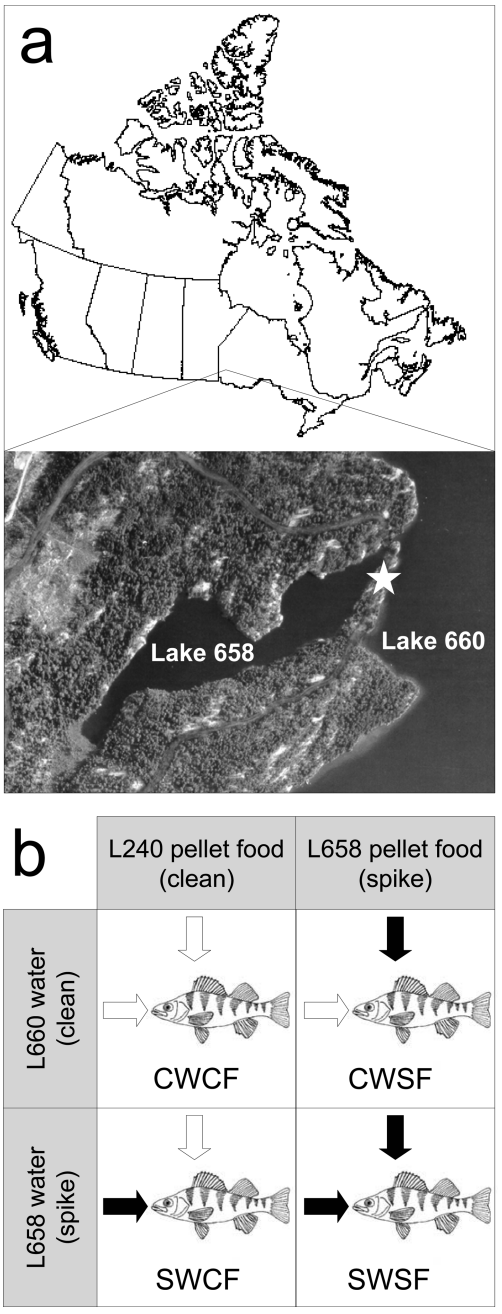

Figure 1. Location (a) and design (b) of experiment. White star on the aerial photograph denotes the location of the fish tanks. We had 3 tanks for each treatment and recovered 2−6 fish from each tank for MMHg analysis, yielding a total of n = 10 fish for each treatment.

Experimental Design. YOY yellow perch were collected from a clean lake (Lake 240) by beach seining, transported to Lake 658, and held in 160-L insulated fiberglass tanks on shore between Lakes 658 and 660. These fish had no previous spike Hg exposure, giving them initial (t_0) spike Hg concentrations of zero. Fish were exposed to one of four treatments designed to separate the sources of spike Hg: clean water + clean food (CWCF); clean water + Hg spiked food (CWSF); Hg spiked water + clean food (SWCF); Hg spiked water + Hg spiked food (SWSF) (Figure 1). Each treatment was replicated three times. Hg spiked water and Hg spiked food were obtained from

Lake 658. After 7 years of Hg isotope additions during the METAALICUS experiment, both water and zooplankton in Lake 658 were "naturally" enriched with spike Hg.[27] Clean water was drawn from Lake 660. All water was filtered through 160-μm mesh to remove larger zooplankton and held overnight in 500-L reservoir tanks prior to being added to the fish tanks. Water temperatures, recorded daily with maximum−minimum thermometers, ranged from 17 to 20.5 °C throughout the study and were not different among treatments (minimum: $F1,100 = 0.86$, $p = 0.47$, $n = 4$; maximum: $F1,100 = 0.63$, $p = 0.60$, $n = 4$).

To avoid contamination of clean water and food that might result from exchange of spike Hg between water and live prey, we created a pelletized fish food using freeze-dried zooplankton collected from Lakes 658 (spike Hg) and 240 (clean) (see ref 28 for a complete description). The resulting food provided consistent dietary Hg exposure to fish throughout the study, while preventing loss of Hg to water that would occur with live prey (e.g., ref 9). We assessed that zooplankton comprise >75% of the diet of YOY yellow perch in Lake 658 and other ELA lakes.[27]

Transfer of Fish and Maintenance of Tanks. Ten YOY yellow perch were euthanized immediately in an overdose bath of 0.25 $g \cdot L^{-1}$ tricaine methanesulfonate (TMS; Syndel Laboratories Ltd., Qualicum Beach, BC, Canada) to determine initial (t_0) fish spike MMHg concentrations. Remaining fish were stocked at a density of 15 individuals per tank. Daily tank maintenance procedures were followed during the experiment (27 d). Pellet food equal to 15% of fish body weight was added to each tank gradually over a 2-h period each day. This feeding regime was designed to satiate fish and promote growth.[29] Following feeding, uneaten food pellets and feces were siphoned out of the tanks. All tanks were drained to half-full daily and refilled with water from reservoir tanks. Due to low initial consumption, beginning on day 7 fish were fed a second time for 1 h after the tanks had been filled. Remaining food was siphoned out at the end of the hour. After 4 weeks of exposure (mid-August to mid-September), fish were euthanized in the field with an overdose of TMS (0.25 $g \cdot L^{-1}$) and immediately placed on ice in a cooler. After transport to the ELA field station fish were measured for fresh length and weight and frozen (−20 °C) in individual WhirlPak bags.

Water Sampling. Water was sampled from each reservoir tank and from one tank of each treatment once per week using a battery-operated pump (CanSun Electronics, Winnipeg, MB, Canada) and clean-hands, dirty-hands protocol[30] (see ref 28 for complete description). Water was also collected from Lake 658 using identical sampling protocol.

To determine whether spike food pellets could have released spike Hg to water during feeding, we conducted an independent trial after completing the main experiment. Two clean tanks were filled with clean water and food pellets were added in the same feeding regime used in the experiment. Water was sampled from each tank 1, 2, and 24 h after adding the food to simulate the typical length of time pellets would be in the tanks (1 and 2 h) and the maximum length of time a pellet would be in the water if it was overlooked during siphoning (24 h). Spike Hg was not detected in any of the water samples collected during this trial.

Processing and Analysis of Samples. All fish collected at the end of the experiment were analyzed for spike MMHg. Samples were handled using mercury clean techniques with Teflon or stainless steel tools.[22] Gut and intestinal tract contents were removed from each frozen fish carcass. Each fish was freeze-dried in a Lyph-lock 12-L freeze-dry system (model 77545, Labconco, Kansas City, MO, USA) until a constant weight was achieved (approximately 72 h). Percent dry weight (i.e., moisture loss) was determined for each individual by dividing dry weight by wet weight. Individual dried fish were ground to a fine powder with an acid-washed glass mortar and pestle. Approximately 0.08 g of the ground tissue from each fish was analyzed for spike Hg.

All water and fish tissue samples were analyzed for spike total Hg (THg) and spike MMHg at Trent University, Peterborough, ON.[31] THg was measured in samples after digestion with HNO_3/H_2SO_4 (7:3 v/v) and heating at 80 °C until brown NO_x gases no longer formed. THg of sample digests was reduced by $SnCl_2$ and determined by inductively coupled plasma−mass spectrometry (ICP-MS) (Thermo-Finnigan Element2) using a continuous-flow cold vapor generation technique. MMHg in samples was solubilized by treatment with 20% (w/v) KOH/MeOH solution at 50 °C and measured after aqueous phase ethylation using $NaBEt_4$. Volatile Hg species were purged and trapped onto Tenax and MMHg was measured after thermodesorption and GC separation using ICP-MS detection (Micromass Platform). Dry weight spike Hg concentrations were divided by the dry weight proportion to yield wet weight spike Hg concentrations. Method blanks and certified reference materials were measured for each batch of samples. Results for TORT-2 (measured mean ± SD, 330 ± 25; certified 270 ± 60) were not statistically different from certified values.

Model Simulations. A bioenergetics-based contaminant accumulation model was used to predict accumulation of spike MMHg by fish. The Wisconsin Fish Bioenergetics version 3.0 model[19] has been used widely to examine contaminant accumulation in fish (e.g., 10, 22, 32) and assumes that fish only accumulate Hg from food.[19] The simulations used identical start (0.8 g) and end fish weights (1.1 g) which were intended to represent growth of an average individual. Input data used for model simulations are described fully in ref 28. Predicted spike MMHg concentrations were compared to mean observed values in the CWSF and SWSF experimental treatments.

Calculations. Spike Hg accumulated from water by fish was estimated using two methods. The first is modified from ref 9 and involves examining the difference in spike Hg concentrations between fish exposed to spike Hg in food and water and fish exposed to spike Hg only in food

$$\%\text{Hg from water} = (([\text{Hg}]_{\text{SWSF}} - [\text{Hg}]_{\text{CWSF}}) \div [\text{Hg}]_{\text{SWSF}}) \times 100\% \quad (1)$$

where $[\text{Hg}]_{\text{SWSF}}$ is the mean wet weight spike Hg concentration ($ng \cdot g^{-1}$) of fish from the SWSF treatment and $[\text{Hg}]_{\text{CWSF}}$ is the mean wet weight spike Hg concentration ($ng \cdot g^{-1}$) of fish from the CWSF treatment.

The second method examines the difference between spike Hg concentrations in fish exposed to spike Hg only through water and fish exposed to spike Hg through both pathways

$$\%\text{Hg from water} = ([\text{Hg}]_{\text{SWCF}} \div [\text{Hg}]_{\text{SWSF}}) \times 100\% \quad (2)$$

where $[\text{Hg}]_{\text{SWCF}}$ is the mean wet weight spike Hg concentration ($ng \cdot g^{-1}$) of fish from the SWCF treatment.

Bioaccumulation factors (BAF) were calculated to describe the differences in Hg concentrations between water and zooplankton

$$\text{BAF} = \log([\text{Hg}]_{\text{f}} \div [\text{Hg}]_{\text{w}}) \quad (3)$$

Table 1. Mean THg and MMHg Concentrations (Range in Parentheses) of the Stable Isotope ^{202}Hg (Spike Hg) in Water and Food Used in Experimental Treatments

component	n	spike THg[b]	spike MMHg[b]	% spike MMHg[c]
water	4	0.031 (0.010−0.073)	0.026 (0.024−0.027)	83.9%
food	2	4.39 (4.19−4.59)	2.16 (2.02−2.29)	49.2%

[a] Clean food and water did not contain detectable levels of spike Hg. Water samples were taken weekly from the spike water reservoir tank. Percent THg that is MMHg is presented for water and food. [b] ng·L^{-1} for water; ng·g^{-1} for food. [c] (MMHg/THg) × 100%.

where $[Hg]_f$ is the concentration of spike MMHg in food (ng·g^{-1} dry weight) and $[Hg]_w$ is the concentration of spike MMHg in water (g·mL^{-1}).

Statistical Analyses. Fish mortality was observed in all tanks, with pulses of high mortality in the first and final weeks of the study likely resulting from stress of transport (first week) and disease (final week). Despite this mortality, 2−6 fish were recovered from each tank. Parametric statistics (one- and two-way ANOVA, t tests) were used to analyze Hg and growth variables using Statistica v5.5 (StatSoft, Inc.) after testing for normality and homogeneity of variance. One-way ANOVA was used to test for differences in mean tank Hg concentrations among tanks within treatments (n = 2−6 fish per tank), and then overall treatment means were determined from the tank means (n = 3 tanks per treatment). A t test was used to compare mean fish mass at t_0 (n = 10) to mass at the end of the study for all treatments combined (n = 40). Two-way ANOVA was used to examine differences in fish growth and spike MMHg concentrations among treatment means. Two-way ANOVA was also used to determine whether food and water accumulation pathways contributed to fish Hg concentrations independently of one another.

RESULTS

Water and Food Spike Mercury Levels. Spike Hg^{2+} was last added to Lake 658 in the fall of 2007, but was detectable in the water column at low levels throughout the 2008 open water season (9 sampling dates between April 3 and November 11, 2008; H. Hintelmann, unpublished data). From weekly sampling of the reservoir tanks, spike THg concentrations in water averaged 0.031 ng·L^{-1} (SEM = 0.014; n = 4), while average spike MMHg water concentration was 0.026 ng·L^{-1} (SEM = 0.001; n = 4) (Table 1). Spike THg was present in food pellets at an average concentration of 4.39 ng·g^{-1} wet weight (SEM = 0.20; n = 2), and the mean spike MMHg concentration in food was 2.15 ng·g^{-1} (SEM = 0.13; n = 2). Log BAFs between water and zooplankton were 5.2 for THg and 4.9 for MMHg. Percent of THg present as MMHg was 83.9% in water and 49.0% in pellet food. Spike THg and MMHg were not detected in water samples from CWSF tanks or in water from experimental tanks in which spike Hg pellet food had sat for 24 h, suggesting that the two uptake pathways were successfully separated in these treatments.

Spike Mercury in Fish. YOY yellow perch showed limited growth during the 27-d study. Average fish body mass increased by 11−28%, but was not significantly different from mean mass at t_0 (t test: $t(48) = -1.79$, p = 0.08). Treatment type did not significantly influence fish growth ($F1,8 = 1.5$; $p = 0.256$) (Table 2).

YOY yellow perch collected from Lake 240 at t_0 did not contain detectable levels of spike MMHg (Figure 2); therefore, all spike Hg present in fish was accumulated from water or food during the experiment. Spike MMHg was detected in whole-body samples of all fish from all treatments except for CWCF (Figure 2). Mean whole-body spike MMHg concentrations in fish did not differ significantly among tanks within treatments ($p > 0.05$ for all treatments). The majority of spike Hg in fish was in the methylated form, with spike MMHg accounting for an average of 93.7% (SEM = 5.6; n = 30) of the spike THg present in all fish.

Spike MMHg was detected in all fish exposed to spike Hg only in water (SWCF) at concentrations significantly higher than those observed in individuals not exposed to spike Hg (CWCF treatment; MMHg not detected) (Figure 2). Exposure to different food and water types (clean or spike Hg) produced significantly different mean whole-body spike MMHg concentrations in YOY yellow perch (two-way ANOVA: food: $F1,8$ = 48.60, $p < 0.05$; water: $F1,8$ = 1440.60, $p < 0.05$) (Table 2). As expected, SWCF fish exhibited the lowest mean spike MMHg concentrations (0.06, SEM = 0.006) among Hg spiked treatments, fish exposed to spike Hg only in food (CWSF) had the next highest average concentrations (mean spike MMHg = 0.26, SEM = 0.010), and SWSF fish showed the highest concentrations

Table 2. Results of Two-Way ANOVA for Growth and Monomethylmercury (MMHg) Concentrations in Young-of-Year Yellow Perch after 27 d of Exposure (n = 3 Tanks Per Treatment)

	growth				MMHg			
	df	MS	F	p	df	MS	F	p
intercept	1	10.047	851.42	0	1	0.279	2232.6	<0.0001
food	1	0.002	0.18	0.68	1	0.006	48.6	0.0001
water	1	0.009	0.72	0.42	1	0.180	1440.6	<0.0001
food*water	1	0.018	1.49	0.26	1	0.001	5.4	0.049

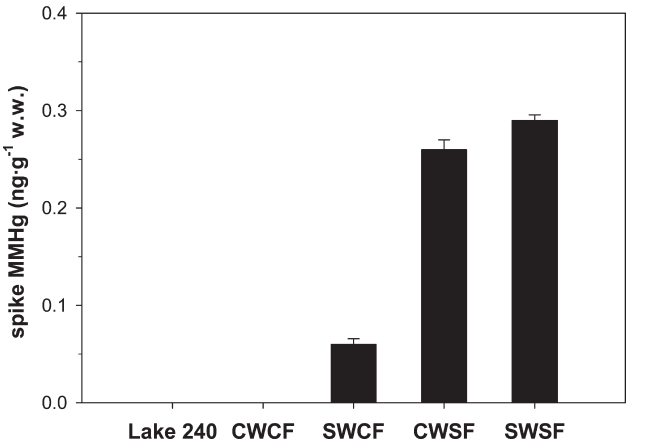

Figure 2. Mean concentrations of spike monomethylmercury (MMHg) in whole YOY yellow perch from Lake 240 (t_0) at the start of the study and after 27 d of exposure to one of four experimental treatments: clean water + clean food; Hg spiked water + clean food; clean water + Hg spiked food; Hg spiked water + Hg spiked food. For each treatment, means (+1 SEM) from replicate tanks (n = 3 per treatment) are presented. A total of 10 fish were analyzed from Lake 240. Spike MMHg was not detected in any fish from Lake 240 or the CWCF treatment.

Table 3. Estimates of Whole-Body YOY Yellow Perch Spike Monomethylmercury (MMHg) Concentrations Predicted by the Wisconsin Model and Mean Concentrations Observed in the Experiment (n = 3 Tanks Per Treatment) for Clean Water + Hg Spiked Food (CWSF) and Hg Spiked Water + Hg Spiked Food (SWSF) Treatments

source	treatment	MMHg[a] (ngg^{-1})	SEM	n	model % difference from experimental concentration
experiment	CWSF	0.26	0.045	3	3.8%
experiment	SWSF	0.30	0.059	3	−11.1%
Wisconsin	SWSF	0.27	-	1	

[a] Mean concentration for experiment; point estimate for Wisconsin model.

(mean spike MMHg = 0.30, SEM = 0.006) (Figure 2). When whole-body spike Hg concentrations were analyzed by two-way ANOVA, the interaction effects were marginally significant for spike MMHg ($F1,8 = 5.4$; $p = 0.049$) (Table 2).

YOY yellow perch accumulated spike Hg directly from water (SWCF treatment). YOY yellow perch exposed to spike Hg in both food and water (SWSF) had 10.3% greater spike MMHg concentrations than fish exposed to spike Hg only in food (CWSF) (eq 1). Using eq 2, fish that received spike Hg from water only (SWCF) had mean spike MMHg concentrations equal to 20.7% of the mean concentrations of fish that received spike Hg from both sources (SWSF).

Model Predictions. The Wisconsin Bioenergetics model assumes all fish Hg is derived from food[19] and is frequently used to predict fish Hg concentrations in nature (e.g., 10, 22, 32). We compared observed spike MMHg concentrations in YOY yellow perch in our study (CWSF and SWSF treatments) to those generated by the Wisconsin model using input data collected during this study (Table 3). The model prediction of YOY yellow perch spike Hg concentration was 4% higher than the mean concentration observed in the CWSF treatment, and 11% lower than the mean concentration of the SWSF treatment.

DISCUSSION

Fish accumulate Hg directly from water and food, but the relative importance of these exposure pathways was unresolved. Over the past decade, water as a source of Hg to fish has been increasingly ignored when estimating or describing Hg accumulation (e.g., 10 and 11). We conducted a field experiment to quantify uptake of MMHg from food and water by YOY yellow perch under seminatural conditions and found that direct accumulation of MMHg from water was significant, accounting for approximately 10% of MMHg accumulation in a period of one month, and up to 20% of total accumulation if sorption onto food is considered. By incorporating isotope enriched Hg as a tracer we were able to establish baseline (t_0) spike THg and MMHg concentrations of zero for all fish and to effectively separate the food and water exposure pathways, two limitations of previous research. Additionally, the use of spike Hg in water and food that had accumulated through a whole-ecosystem loading study greatly increased the realism and applicability of this field trial.

Spike Hg Accumulation. The presence of spike MMHg in YOY yellow perch that were only exposed to spike Hg in water (Figure 2) indicates that fish accumulated Hg directly from water. In this study, fish showed a range of spike Hg uptake from food and water from 0 to 100%, depending on treatment type. Concentrations of spike MMHg were 10% greater for YOY yellow perch exposed to spike Hg in food and water (SWSF) compared to the food-alone treatment (CWSF; eq 1). This estimate was confirmed with the use of a published biokinetics model,[14] which suggested fish would accumulate 8−13% of their Hg from water in this experiment. The estimate of 10% MMHg accumulation from water in this study is consistent with results of laboratory, field, and modeling studies. In a modeling exercise, ref 33 indicated that model estimates of Hg in yellow perch were approximately 10% lower than observed values. Similarly, bioenergetics modeling completed by ref 34 indicated that food was responsible for over 90% of MMHg uptake in yellow perch and walleye (*Sander vitreus*) in oxic freshwaters. In both cases, it was suggested that the "missing" MMHg was derived from water. Further, ref 9 concluded that accumulation of aqueous Hg by finescale dace represented at most 15% of total uptake. The high consistency between previous studies and the present experiment suggest that 10% is a robust estimate of waterborne Hg uptake in a range of fish species. The improvements in experimental design now possible through the incorporation of enriched stable isotopes of Hg strengthen the conclusions drawn in this study.

Fish that were exposed to spike MMHg in water alone (SWCF) accumulated approximately 20% of the MMHg observed in fish exposed to spike in food and water (SWSF). This value is double the estimate for waterborne uptake derived in eq 1, and may be due, in part, to sorption of MMHg to clean food particles prior to ingestion. We specifically kept our feeding period short (2 h) to minimize potential transfer of waterborne MMHg onto food pellets, but the greater uptake of MMHg by YOY yellow perch when exposed only to spike MMHg in water suggests that sorption onto food particles likely occurred in our study. The inability to distinguish between fish MMHg accumulation from water and short-term sorbtion onto particles has been identified in previous research.[14] Although this may inflate the apparent importance of water as a source of Hg, MMHg sorbed to the surface of the food pellets originated in water and remained on the surface of the food pellet. It follows that this sorbed fraction of MMHg may represent waterborne MMHg that is accumulated through the gut rather than the gills, however, water remains the source of this MMHg to the fish. The rapid sorbtion of MMHg onto particles may be an important route through which waterborne MMHg is accumulated by aquatic biota, separate from respiration.

Sorption of spike MMHg onto clean food particles may have yielded the marginally significant interaction observed in the two-way ANOVA of MMHg concentrations. This suggests that the food and water accumulation pathways were not completely separated in this study. Previous research has suggested that accumulation of Hg from food and water are additive,[7,12] and that Hg follows first order kinetics once inside the body of a fish,[17] acting as a single mass of Hg. Although accumulation from food and water appear to be additive in the present study, our experimental design did not allow us to determine that definitively.

The short duration of this study coupled with environmentally relevant (i.e., low) concentrations of spike Hg in water and food meant that concentrations of spike Hg in experimental fish were correspondingly low. However, patterns of spike MMHg accumulation were consistent with previous research (e.g., 9, 14). Fish that received spike MMHg from both sources had significantly

higher concentrations than those exposed to spike MMHg only in food, suggesting that accumulation via diet could not account for all spike MMHg accumulation in SWSF fish. Accordingly, fish exposed to spike Hg from water alone accumulated low, but detectable, concentrations of spike MMHg in their tissues. Many studies (e.g., 7, 13) and Hg accumulation models (e.g., 8, 33) published in the 1970s to 1990s considered waterborne Hg accumulation an important contributor to overall fish Hg levels. Our results are consistent with this mindset, and we caution against the complete disregard of water as a source of Hg to fish, which has been the trend in accumulation studies and models over the past two decades.

Applicability to Natural Populations. Previous Hg transfer studies have used elevated Hg concentrations in food and water[7,35] which may yield results that are not applicable to fish in lakes that do not have large point source inputs of Hg. We used low levels of spike Hg in pellet food and water designed to mimic environmental Hg concentrations in "pristine" regions. For example, the Experimental Lakes Area (ELA) receives <4 μg THg $m^{-2}\cdot yr^{-1}$ ambient deposition.[26] Lake 658 at the ELA, has ambient water THg concentrations of 1.7 $ng\cdot L^{-1}$, water MMHg concentrations of 0.2−0.6 $ng\cdot L^{-1}$, and dry weight zooplankton concentrations of 100−500 ng MMHg $\cdot g^{-1}$.[26] Similar water Hg concentrations have been observed in New York State lakes: THg concentrations of 0.3−7.7 $ng\cdot L^{-1}$ and MMHg concentrations of 0.03−3.6 $ng\cdot L^{-1}$.[36] We also maintained a realistic bioaccumulation factor (BAF) between water and food (4.9 for MMHg) that was fairly consistent with log BAF of ambient MMHg in the study lake (5.8, M. Paterson, unpublished data) and elsewhere.[37] Realistic exposure levels increase the applicability of our results to natural populations of small fish exposed to low levels of Hg contamination.

To maintain a natural food source but avoid the complication of Hg transfer from food to water that can occur when using live prey[9] we constructed a pelletized food containing zooplankton collected from clean and spike Hg lakes. Although some spike MMHg may have sorbed to clean food pellets and thus been ingested by the fish, we are confident that the amount of spike MMHg transferred in this way was far less than we would have observed with live prey. To avoid this complication, it would be advantageous for future studies to test for sorption of MMHg onto food pellets. The use of zooplankton provided a natural exposure ratio of MMHg:THg to fish (49%), similar to those observed elsewhere (30−90%,[37] 24−54%[25]). Overall, we were able to closely mimic exposure conditions observed in nature. To our knowledge, this is the first Hg accumulation study to incorporate natural zooplankton into pellet food in this manner.

Low growth rates exhibited by YOY yellow perch in this study may have exerted an influence on our results. Although the experiment took place under optimal temperatures for growth, and YOY yellow perch were essentially allowed to feed *ad libitum*, fish mass did not increase significantly during the ∼1 mo period (11−28%). It is possible that these estimates of growth are inflated compared to actual increases in mass, because many of the small fish died prior to the conclusion of the study. It is possible that the fish that survived until the end represented individuals that began the study with greater than average mass. This would mean the apparent % increases in mass were greater than the actual increases. Nonetheless, observed growth patterns are typical of YOY yellow perch in Lake 658, with percent increase in mass ranging from 1% to 35% over a 1 mo period (P. Blanchfield, unpublished data). Similarly, ref 9 observed limited growth and many cases of weight loss over a 32-day experiment designed to quantify ambient Hg uptake by finescale dace and ref 35 reported limited growth in a 30-day study of Hg accumulation from food and water by carp (*Ctenopharyngodon idella*). Neither study suggests that lack of growth influenced their estimates of relative Hg accumulation from food and water, and both indicate that water acts as a source of Hg to fish, however, additional research may be necessary to better assess accumulation under various growth scenarios.

To more fully understand the potential importance of water as an exposure pathway to fish, we must still resolve the seasonality of exposure. One study[12] suggested that accumulation of Hg from water is of minimal importance in warm summer months, but becomes more dominant in fall and winter with declining consumption and activity rates. This suggestion is consistent with observed increases in fish MMHg concentrations between fall and spring (e.g., 38), which may result in part from increased accumulation of Hg from water during winter. Additionally, dramatic fall increases of MMHg concentrations of small fish commonly coincide with lake destratification when MMHg-rich waters are released from the hypolimnion[38,39] and may result from direct aqueous uptake of newly available waterborne MMHg. Our study provides evidence that fish accumulate Hg directly from water under late-summer conditions. Further experimental trials conducted under a winter thermal regime would enhance our understanding of the overall importance of waterborne Hg accumulation by fish in north temperate lakes where MMHg concentrations in fish remain a major concern.

Model Predictions. We used the Wisconsin Fish Bioenergetics model, a widely used bioaccumulation model that assumes all fish Hg is derived from food, to predict YOY yellow perch Hg concentrations for treatments that included exposure to spike Hg only in food and exposure to spike Hg in food and water, which is how it is used when applied to natural conditions. As expected, the model accurately predicted spike Hg concentrations observed in fish exposed to Hg only in their food (CWSF treatment; Table 3), but when compared to fish exposed to spike Hg in both sources (SWSF treatment), predicted concentrations were 11% lower than concentrations measured experimentally. Because experimental conditions were consistent among treatments and all input parameters were known, exclusion of water as a source of Hg in the model was the root of the underestimation. Previous modeling studies have observed similar results, with model estimates falling approximately 10% short of experimentally derived Hg concentrations (e.g., 33, 34). The convergence in underestimation of THg concentrations in fish among modeling excercises compared to the present study provides another line of evidence that current models could be improved by accounting for 10% accumulation directly from water. This study highlights the importance of subjecting models to frequent evaluations and revision, particularly as new experiments and field studies clarify details of Hg transfer and accumulation. Many existing accumulation models are based on a limited number of laboratory studies, and it would be advantageous for models to be updated with new information as it arises, especially field-based research. Isotope enriched Hg provides an excellent tool to test and calibrate bioaccumulation models. For example, a field-based Hg elimination study that used isotope enriched Hg concluded that two Hg accumulation models overestimated the rate of Hg loss in age-1 yellow perch.[22] It is evident from the results of the present study that accumulation may also not be represented accurately.

The present study identifies the importance of including both food and water as sources of Hg to fish in bioaccumulation modeling exercises. It may not be appropriate to assume that fish will consistently derive specific proportions of Hg from water and food because local environmental conditions and species-specific parameters will influence Hg accumulation; however, it is important to recognize that both food and water contribute to Hg present in fish tissues and this should be reflected in Hg bioaccumulation models. Several existing Hg bioaccumulation models include water as a source of Hg to fish, indicating that food is a more influential source, but uptake of Hg from the dissolved phase should not be discounted.[8,33,40]

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