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Low-Level Experimental Selenite Additions Decrease Mercury in Aquatic Food Chains and Fish Muscle but Increase Selenium in Fish Gonads

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Abstract We investigated whether low-level addition of selenium (Se) could decrease mercury (Hg) in freshwater fish without imposing Se toxicity. Using a regression design, selenite was added to large mesocosms in a lake to achieve target concentrations ≤1.6 μg/L. ^{198}Hg (spike Hg) was added to mesocosms to determine changes in Hg bioaccumulation. Adding Se decreased spike total Hg (THg) in fish muscle, ambient THg in fish liver, and bioaccumulation of spike THg in muscle and spike methylmercury (MeHg) in zooplankton and *Chironomid* larvae relative to controls. Se decreased Hg in the food web but not in water, indicating that the dominant effect of Se on Hg cycling occurs in the food web. Concentrations of Se in gonads of fish were positively correlated with Se concentrations in water but did not exceed reproductive toxicity thresholds after 8 weeks. We conclude that low-level addition of Se decreases MeHg bioaccumulation and increases Se in gonads of fish; however, additions of Se to freshwater systems to decrease Hg in fish should be treated with caution because Se in fish gonads were likely to exceed toxic concentrations if exposed to increased Se for a longer period of time.

Mercury (Hg) contamination is the cause of concern about human consumption of fish and shellfish around the world. Increased Hg concentrations have resulted from many point-source additions of the element as well as widespread deposition due to fossil fuel burning and other human activities. Adding selenium (Se) to aquatic systems has been proposed as a mitigative solution; however, there are concerns about the risk of Se toxicity at increased concentrations. Se additions have been shown to decrease Hg concentrations in fish (Turner and Rudd 1983; Paulsson and Lundbergh 1991), and Hg and Se have been shown to interact in aquatic systems (Southworth et al. 1994; Chen et al. 2001). However, observed concentrations of Se ≤ 100 μg/L have been associated with toxic effects from Se. In contrast, Se protects against Hg exposure for people who eat fish (Raymond and Ralston 2004), and Se can protect fish and their wildlife consumers from the toxic effects of Hg (Peterson et al. 2009). We wanted to determine whether low-level Se additions (maximum 1.6 μg/L) could significantly decrease Hg in fish without reaching Se levels that could cause toxicity.

The processes that decrease Hg concentrations with Se additions are not clear. Se may decrease rates of Hg methylation in aquatic environments (Chen et al. 1997; Jin et al. 1999) or directly modify Hg bioaccumulation (Turner and Rudd 1983; Turner and Swick 1983) and redistribute or increase loss of Hg from tissues of fish (Bjerregaard et al. 1999). In contrast, the toxicity of Se has been well studied, and the mode of toxicity is better understood (Janz et al. 2010). Se is essential for the production of amino acids and activation of thyroid hormone, but excessive

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exposures cause deformities in the offspring of fish because Se substitutes for sulphur during protein synthesis. Fish require 0.1–0.5 μg/g Se in their diet (Hodson and Hilton 1983), but dietary concentrations >3 μg/g can cause toxicity (Lemly 1997). The accepted threshold for Se toxicity is 17–24 μg/g (dry weight [dw]) in fish tissue (Janz et al. 2010).

We used additions of Se to large mesocosms in a lake in northwestern Ontario to test whether low-level addition of Se could decrease Hg in fish. We hypothesized that additions ≤1.6 μg/L of Se would decrease Hg concentrations in muscle of fish. We also wanted to assess whether low-level additions of Se would result in toxic Se levels in fish gonads because the main mechanism of toxicity of Se in fish is maternal transfer of Se to eggs during vitellogenesis (Janz et al. 2010).

Materials and Methods

Experimental additions of Se were made to mesocosms in a boreal lake to assess the responses of Hg in the food chain and Se in gonads of fish. The experiment was performed in Lake 239 at the Experimental Lakes Area (ELA), which is located approximately 55 km east–southeast of Kenora, northern Ontario (49°40′ N, 93°43′ W). The area is predominantly jack pine forest intermixed with white birch and is characterized by numerous small lakes on glacially scoured bedrock. Lake 239 has a circum-neutral pH and is oligotrophic. Mesocosms were located in a protected bay with sandy sediments and were each 10 m in diameter with a maximum depth of 2.8 m. Mesocosms were suspended from floating rings, and the walls were constructed of woven polylaminated plastic that was open to the sediments. The calculated volume of each mesocosm was approximately 196,000 L on the basis of mean depth. Mesocosms were deployed on May 30, 2005.

Selenite was selected because it is the most affordable form of Se to apply, is readily available, has been shown to be effective, and is converted to forms of Se that are available to biota after adding to lake water. Selenite is therefore the likely form of Se that would be used for a large-scale application. Using a regression model, Se was added to each of six randomly assigned mesocosms to achieve concentrations of 0, 0.1, 0.2, 0.4, 0.8, and 1.6 μg/L of selenite (Na_2SeO_3). Concentrations of Se were known to be <0.1 μg/L in surrounding lakes on the basis of preliminary sampling (Mailman 2008). An electric trolling motor was used to mix the treatments into the water after sunset on June 22, 2005. There was a positive relationship between mean measured and intended Se concentrations ($r^2 = 0.98$, $p < 0.0001$), and resulting Se concentrations were close to intended concentrations (Mailman 2008) based on the determination of Se concentrations in the mesocosms every 2 weeks after treatment.

A stable isotope of Hg (^{198}Hg; 93.53 % isotope; hereafter referred to as spike Hg) as mercuric chloride was added as a tracer that could be analyzed separately from ambient (or background) Hg. This added Hg provided a measure of newly accumulated Hg by biota during the experiment. Approximately 10 times the mean annual atmospheric deposition of Hg at the ELA was added to all six mesocosms (5.8 mg or 0.3 μg/L). The isotopic Hg was dissolved in nitric acid (5 %) and mixed with approximately 500 mL of water from Lake 239 in Teflon bottles the night before treatment addition according to Paterson et al. (2006).

Analyses of Se and ambient and spike total Hg (THg) and MeHg were performed on muscle, liver, gills, and brains of fish to examine tissue distributions, possible entry routes, and dynamics of Hg and Se in tissues. Gonads of fish were analyzed for Se to determine the risk of toxicity. Zooplankton, *Chironomid* larvae, and periphyton were analyzed for Se and MeHg.

Sample Collection

Periphyton

On July 10, 2005, three strips of 5 cm–wide wall material were suspended from a floating board in each mesocosm, and each strip was weighted at the bottom. On August 16, 2005, periphyton strips were removed using clean gloves and placed in new polyethylene bags in a cooler on ice. In the laboratory, periphyton was scraped and rinsed from strips and frozen in polyethylene bags.

Zooplankton

A pretreatment sample of zooplankton was obtained from the mesocosms, and they were sampled every fourth week after treatments were added. At the experiment's initiation, equal amounts of zooplankton captured from Lake 239 using a 150 μm mesh net were added to each mesocosm to augment the zooplankton population. Subsequent zooplankton samples were collected within all mesocosms using horizontal tows of a 150 μm mesh Wisconsin net. Samples with >10 % algae, sand, or suspended particulate matter were rejected.

Macroinvertebrates

Chironomid larvae were present in all mesocosms and were collected using a Ponar dredge. Samples were sorted after sieving through 12.5 mm to 250 μm mesh. Pretreatment samples were collected adjacent to mesocosms, and final

samples were collected from inside each mesocosm after all other sampling was completed at the end of the experiment.

Fish

Mesocosms were stocked with yellow perch (*Perca flavescens*) that were taken from Lake 239. Before the experiment, mesocosms were fished extensively to remove fish captured after deployment of the mesocosms. Yellow perch were then collected from Lake 239 using hoop-and-trap nets and beach seining. A subsample of 30 fish was randomly taken in June for pretreatment analyses. Fish added to mesocosms were killed with MS-222, measured for fork length and weight, and tagged with a subcutaneous decimal-coded wire cheek tag. Addition of fish to mesocosms continued for 3 weeks after treatment addition to maintain 18 fish/mesocosm. The duration of exposure of an individual fish ranged from 5–8 weeks. Dead fish were removed every 2–4 days. There was no indication of mortality being related to the dose of Se. Due to fish mortality, final sample sizes of fish were variable: treatment 0, $n = 4$ (two of these were found floating, and no liver was available); treatments 0.1 and 0.2, $n = 8$; treatment 0.4, $n = 16$; treatment 0.8, $n = 4$; and treatment 1.6, $n = 2$.

Fish were collected from mesocosms using baited minnow traps, small mesh gill nets, seine net, and by snorkeling and SCUBA diving with dip nets. Fish were killed by an overdose of MS-222. Gonads, liver, kidneys, brain, gills, and subsamples of skinless dorsal muscle were removed and frozen individually. Data for all compartments are from the final sample date unless otherwise specified.

Analyses

After freeze-drying samples of sediment, periphyton, zooplankton, invertebrates, and fish tissues, subsamples were weighed on a Perkin Elmer microbalance (2–10 mg for Hg analyses and 10–20 mg for Se analyses). An acid-rinsed stainless steel scoop was used to transfer the material into trace metal–clean weigh boats. Subsamples were placed in Teflon vials cleaned in hot nitric acid. Vials were stored in sealed polyethylene bags.

Analyses of Hg

Analyses of THg in fish was by cold-vapour atomic absorption spectrometry and of THg and MeHg in other media by cold vapour atomic fluorescence spectrometry as described by Paterson et al. (2006) with the following modifications. Using isotope-dilution analyses, each sample of tissue was spiked with 100 µL of 60 ng/mL of internal Hg standard (2.3 ng/mL of $^{201}Hg^{2+}$). Tissues were digested with 5 mL of HNO_3 and H_2SO_4 (7:3) at 120 °C for 4 h. For MeHg analyses, tissues were digested with 5 mL of KOH–methanol, and samples were heated for 24 h at 50 °C and then diluted to 20 mL with deionized water. The limit of detection (LOD) for ambient MeHg in zooplankton and *Chironomid* larvae was 0.02–1.0 ng/g (dry weight [dw]) and in fish was 1.0 ng/g (dw) for THg and 2.0 ng/g (dw) for MeHg. For spike Hg, the LOD was 0.5 % of the ambient Hg concentration in each sample.

Analyses of MeHg in filtered water used 50 mL of samples to which the following were added: 20 µL of 1 ng/mL of ^{201}MeHg solution, 500 µL of 9 M H_2SO_4, and 200 µL of 20 % KCl. Samples of filtered water were distilled at 140 °C for 5–7 h. Thereafter, sodium tetraethyl borate was used to ethylate MeHg in the distillate, which formed volatile Hg species that were purged from solution and adsorbed to Tenax traps at room temperature. Species of Hg were separated by gas chromatography (Horvat et al. 1993; Hintelmann and Evans 1997). No sample was added to digestion blanks. Digestion and filter blanks were also analyzed. For MeHg, the LOD was 0.02 ng/L in filtered water. Spike MeHg LOD was 0.5 % of the LOD for ambient MeHg. Certified reference material (CRM–DORM 2 National Research Council of Canada dogfish muscle for THg and oyster tissue for MeHg) was prepared and digested in the same manner as samples, and results were not significantly different from expected values.

Analyses of Se

Se was detected by atomic fluorescence hydride generation spectroscopy using a PS Analytical Millennium Excalibur Model 10.005. To measure total Se (TSe), slight modifications were made to a previous method (Cutter and Bruland 1984): BrCl was added as an auxiliary oxidant instead of potassium persulfate (Brindle and Lugowska 1997). Samples were heated for 15 min before adding HCl to 4N, and then these samples were cooled, which yielded consistent results regardless of subsequent heating.

To 9.35 mL of each sample of filtered water, 200 µL of BrCl was added. The solution was heated at 93–97 °C for 15 min and then cooled. It was adjusted to 4N HCl, reheated in the same manner, cooled, and then analyzed. Tissues (0.05 g) were digested with 5 mL of nitric-sulphuric acid (muscle and biota = 1:2.5; liver and fatty tissue = 7:3) for 1 h at room temperature. Tissues were then heated to 150 °C for 6 h, cooled, and then made up to 10 mL with deionized water. To measure TSe, 8.35 mL of deionized water and 200 µL of BrCl were added to 1 mL of digest. This solution was capped tightly and sat overnight at room temperature. It was then heated to 93 °C to

97 °C for 15 min, cooled, adjusted to 4N HCl, reheated in the same manner, cooled, and then analyzed.

Each analytical run included two matrix spikes and their duplicates, a duplicate of one sample, and duplicates of CRM. The CRM for water—TM-28.2, lot 603 (Environment Canada National Water Research Institute in Burlington, Ontario)—contains 3.6 μg/L of Se ± 0.96 (SD). For samples collected in August, we measured 3.24 ± 0.12 μg/L (90 % recovery).

All standards, blanks, and CRMs were treated the same way as those for samples, including digestion with BrCl. Measurements of Se concentrations in samples were repeatable. Triplicate analysis of water had a mean recovery of 106.3 %. CRM for invertebrates, including muscle, gills, and gonads, was DORM-2. Its certified valued is 1.4 ± 0.09 mg/kg. In references for the following matrices, we measured zooplankton = 1.35 mg/kg ± 0.09 (96.4 % recovery [4 runs]), *Chironomid* larvae = 1.29 mg/kg ± 0.14 (92.1 % recovery [4 runs]), gills = 1.45 mg/kg ± 0.05 (103.6 % recovery [8 runs]), and gonads = 1.30 mg/kg ± 0.06 (92.9 % recovery [8 runs]). CRM for liver was DOLT-2–certified at 6.06 mg/kg ± 0.49 and measured 5.67 mg/kg ± 1.25 (93.6 % recovery [6 runs]). CRM for brain was LUTS-1–certified at 0.641 mg/kg ± 0.054 and measured 0.66 mg/kg ± 0.07 (103 % recovery [8 runs]).

Analyses of $\delta^{13}C$ and $\delta^{15}N$

Analyses of $\delta^{13}C$ and $\delta^{15}N$ took place at the National Hydrology Research Centre Stable Isotope Laboratory in Saskatoon, Saskatchewan, by standard methods using a Carlo Erba elemental analyzer followed by a continuous-flow isotope ratio mass spectrometer. Freeze-dried subsamples of 1 mg ± 0.2 mg were combusted to convert organic carbon (C) to CO_2 and nitrogen (N) to N_2. All samples are reported against Vienna Pee Dee Belemnite using synthetic gelatine and bowhead whale baleen for $\delta^{13}C$ and diatomic N in air for $\delta^{15}N$. Results are reported as $^{o}/_{oo}$, which is related to the reference material by the equation: $\delta X = [(R_{sample}/R_{standard}) - 1] * 1000$ where $X = {}^{13}C$ or ^{15}N and $R = {}^{13}C/{}^{12}C$ or $^{15}N/{}^{14}N$. Total precision was ± 0.2 $^{o}/_{oo}$ for both C and N.

Data Analyses

Bioaccumulation factors (BAFs) for periphyton, zooplankton, *Chironomid* larvae, and yellow perch tissues were calculated using the dry-weight concentration (ng/g) of MeHg (or THg in tissues of fish) divided by the concentration of MeHg in filtered water (ng/mL). BAFs account for both differences in the dissolved pool of MeHg available for uptake into the base of the food web as well as those in trophic transfer among mesocosms, and both could have been changing in this experiment.

Simple linear regressions modeled the relationship between spike Hg or MeHg concentrations and BAFs in biota and TSe concentrations in filtered water. Concentrations of Se in filtered water were used as the independent variable to most accurately approximate Se concentrations to which biota were exposed. All data were $\log_{10}$-transformed before analysis using statistical analysis software (SAS) version 9.1. Multiple regressions were used to analyze the relationships between ($\log_{10}$) BAFs of Hg or MeHg as a function of TSe concentrations in filtered water.

Data for $\delta^{13}C$ and $\delta^{15}N$ for samples of surface sediment, periphyton, and muscle of yellow perch were statistically analyzed using analysis of variance (ANOVA) with Tukey multiple comparisons for data sets with equal sample sizes and general linear model for unbalanced data when sample size varied; however, results are pseudoreplicated, so statistical results should be treated with caution. Corrections for multiple comparisons were not made. When measurements were below the LOD, which occurred in one sample of periphyton, half the detection limit was used.

Results

Bioaccumulation of Ambient and Spike THg, MeHg, and TSe

Se additions significantly decreased bioaccumulation of new Hg in the food chain. After 8 weeks, spike Hg was detected in muscle of all yellow perch (Fig. 1), with 95–97 % of the THg present as ambient THg. There was no statistically significant relationship between ambient THg concentrations in muscle and Se concentrations in filtered water ($r^2 = 0.42$; $p = 0.16$); however, concentrations of newly accumulated spike THg in muscle correlated negatively with Se concentrations in filtered water ($r^2 = 0.90$, $p = 0.004$). Differences were not due to differences in diet among mesocosms because analyses of stable isotopes of C and N indicated that the diets of perch did not vary significantly.

In liver of yellow perch, 48–63 % of the THg was present as ambient THg, and the remainder was spike THg (Fig. 1). Concentrations of spike THg were 10–20 times greater in liver than those in muscle. In contrast to results for muscle, ambient THg concentrations in liver of yellow perch were negatively correlated with TSe concentrations in filtered water ($r^2 = 0.80$, $p = 0.02$), whereas spike THg concentrations in liver were not ($r^2 = 0.62$, $p = 0.06$). Gills and brains also contained ambient and spike THg; however, neither were related to Se concentrations in filtered water.

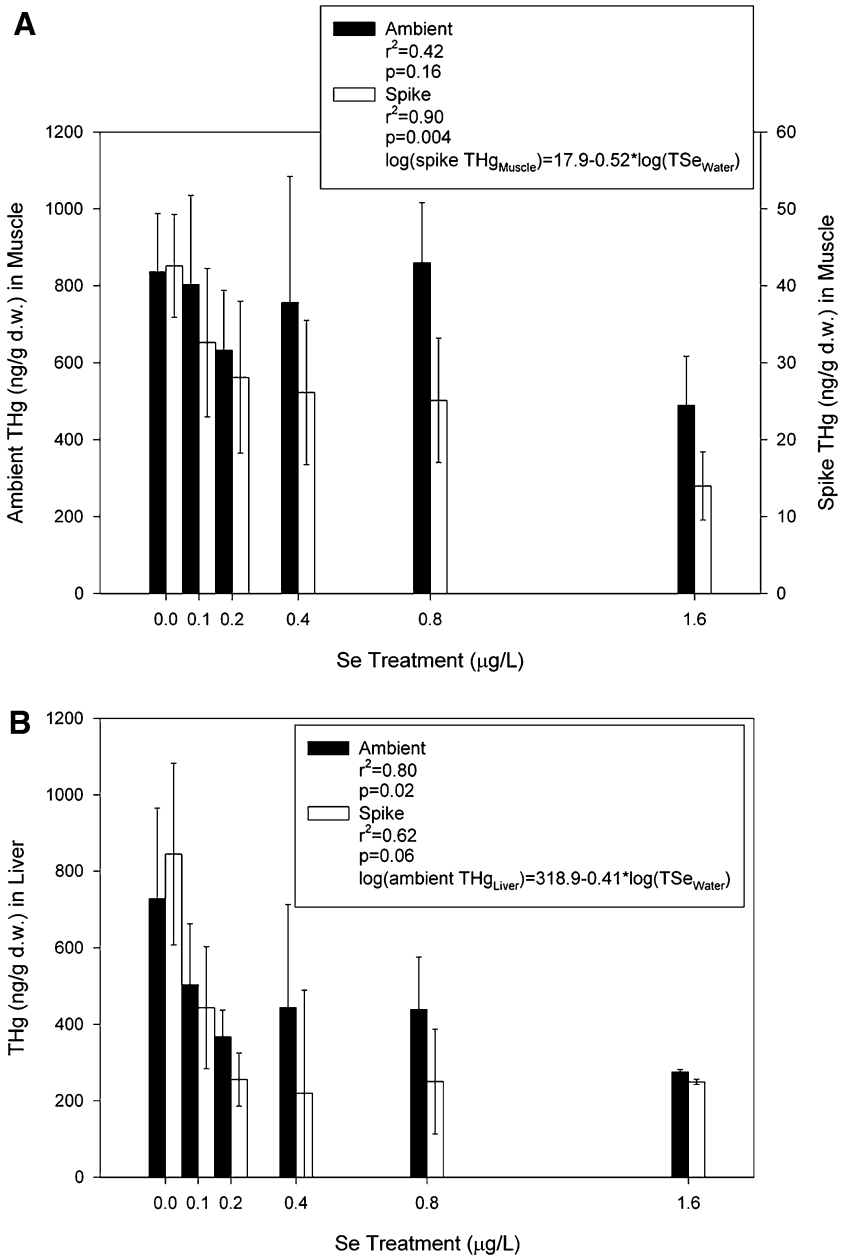

Fig. 1 Ambient and spike THg concentrations in muscle and liver of *yellow* perch versus Se treatment. The ambient THg axis is on the *left*, and the spike THg axis is on the *right*. *Error bars* = 1 SD. The regression equation was calculated using actual concentrations of spike THg in relationship to mean Se concentrations in water. The *bars* were graphed at intended Se concentrations for ease of visibility

Spike THg was present in biota as spike MeHg. Ambient and spike MeHg in muscle of fish was measured in three mesocosms (Fig. 2), and slightly <100 % of ambient THg was present as ambient MeHg, whereas approximately 65 % of the spike THg in muscle was present as spike MeHg. There was more ambient MeHg than spike MeHg in muscle of fish (Fig. 3), zooplankton, *Chironomid* larvae, and periphyton. Spike MeHg accounted for 0.06–0.08 %, 10–20 %, 3–10 %, and 9–19 % of ambient MeHg, respectively.

Spike MeHg was incorporated into all compartments of the food web. Ambient MeHg concentrations in zooplankton ranged from 45 to 160 ng/g after 8 weeks and were not related to TSe concentrations in filtered water ($r^2 = 0.53$, $p = 0.10$ [Fig. 3]). 8 weeks after treatment addition, spike MeHg concentrations in zooplankton decreased with TSe concentrations in filtered water; however, this relationship was not statistically significant ($r^2 = 0.62$, $p = 0.06$). Ambient and spike MeHg concentrations in *Chironomid* larvae and periphyton were not related to TSe concentrations in filtered water.

Comparison of BAFs among mesocosms suggests that Se modified the accumulation of spike Hg in the food web. BAFs ranged from 4.5 to 5.2 for periphyton, 5.2 to 5.9 for *Chironomid* larvae, and 5.7 to 6.5 for zooplankton (Fig. 4). BAFs of spike THg in muscle of yellow perch ranged from 3.2 to 3.7 (Fig. 5), whereas those in liver, gills, and brain ranged from 4.3 to 4.9, 3.5 to 3.7, and 3.7 to 4.2,

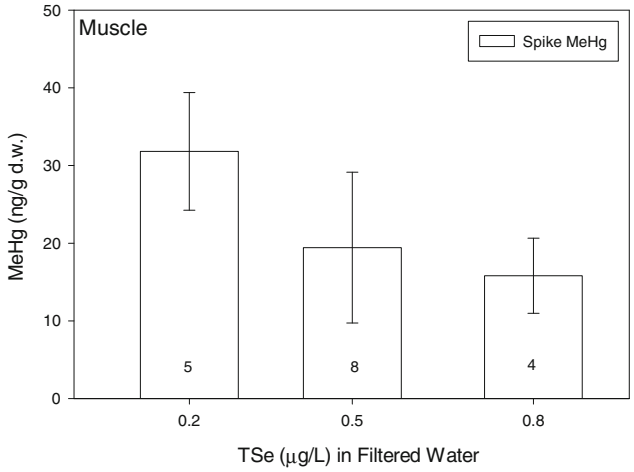

Fig. 2 Concentrations of MeHg in muscle of perch versus TSe concentrations in filtered water. Each *bar* represents mean ± 1 SD. Sample sizes are noted above groups of bars. This relationship was not significant

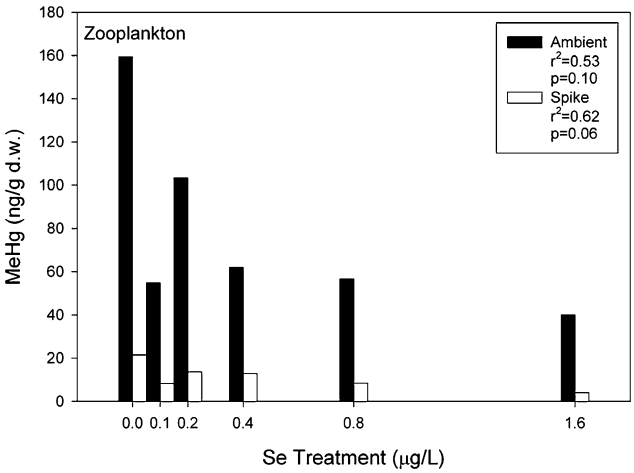

Fig. 3 Ambient and spike MeHg concentrations in zooplankton versus Se treatment in August. Each *bar* represents one sample

respectively. Relationships of BAFs of spike MeHg in zooplankton ($r^2 = 0.75$; $p = 0.02$) and Chironomid larvae ($r^2 = 0.66$; $p = 0.05$) with TSe concentrations in filtered water were statistically significant, whereas in periphyton they was not ($r^2 = 0.21$; $p = 0.36$). The relationship between BAFs of spike THg in muscle of yellow perch as a function of TSe concentrations in filtered water was statistically significant ($r^2 = 0.73$; $p = 0.03$); however, in other tissues it was not (liver: $r^2 = 0.41$; $p = 0.17$, gills: $r^2 = 0.54$; $p = 0.10$; brain: $r^2 = 0.09$; $p = 0.56$).

Se in Gonads of Yellow Perch Was Related to Se in Water

In 98 % of yellow perch that had developed gonads, detectable concentrations of Se were measured after

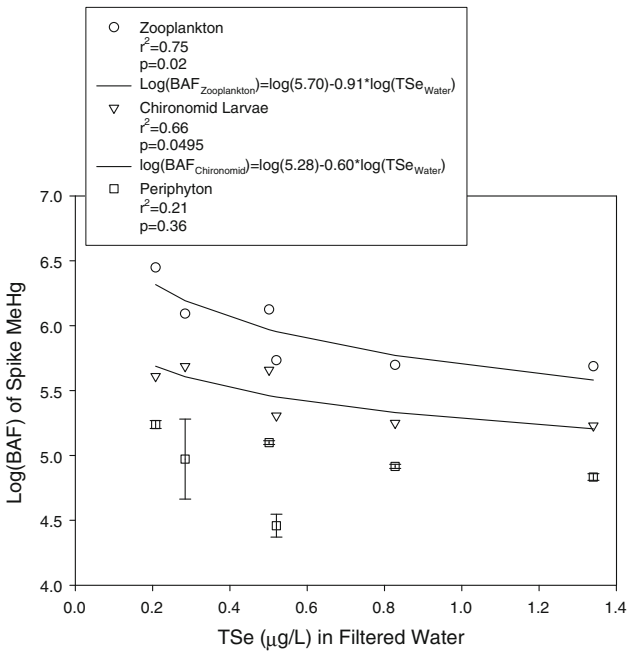

Fig. 4 BAFs of spike MeHg in zooplankton, *Chironomid* larvae, and periphyton as a function of TSe concentrations in filtered water in August. Each data point for zooplankton and *Chironomid* larvae represents one sample, and each data point for periphyton represents the mean of three samples with 1 SD

8 weeks of exposure. Average concentrations in gonads ranged from 3 to 12 µg/g (dw [Fig. 6]). Concentrations of TSe in gonads of yellow perch were significantly correlated with TSe concentrations in filtered water ($r^2 = 0.97$; $p = 0.0002$). Concentrations of TSe in gonads of yellow perch positively correlated with those in muscle ($r^2 = 0.67$; $p < 0.0001$; $\log[TSe_{Gonads}] = 0.352 + 0.772 * \log[TSe_{Muscle}]$; data not shown).

Mean concentration of Se in gonads in treatment 0.8 was 8.9 ± $p = 0.032$.2 µg/g (maximum 11.7 µg/g); in treatment 1.6, the mean concentration was 13.1 ± 5.6 µg/g (maximum 17.0 µg/g). In addition, Se in liver of fish was significantly related to Se additions.

Discussion

Adding Se Decreased Ambient Hg Concentrations in Liver and Spike Hg Concentrations in Muscle of Yellow Perch

Low-level addition of Se decreased the bioaccumulation of new Hg into fish muscle and liver. In treatments with greater Se concentrations, ambient Hg concentrations in liver and spike Hg concentrations in muscle were lower compared with controls. 8 weeks after Se additions, 1.3 µg/L of Se decreased mean spike or recently methylated Hg concentrations in muscle of yellow perch by 59 %

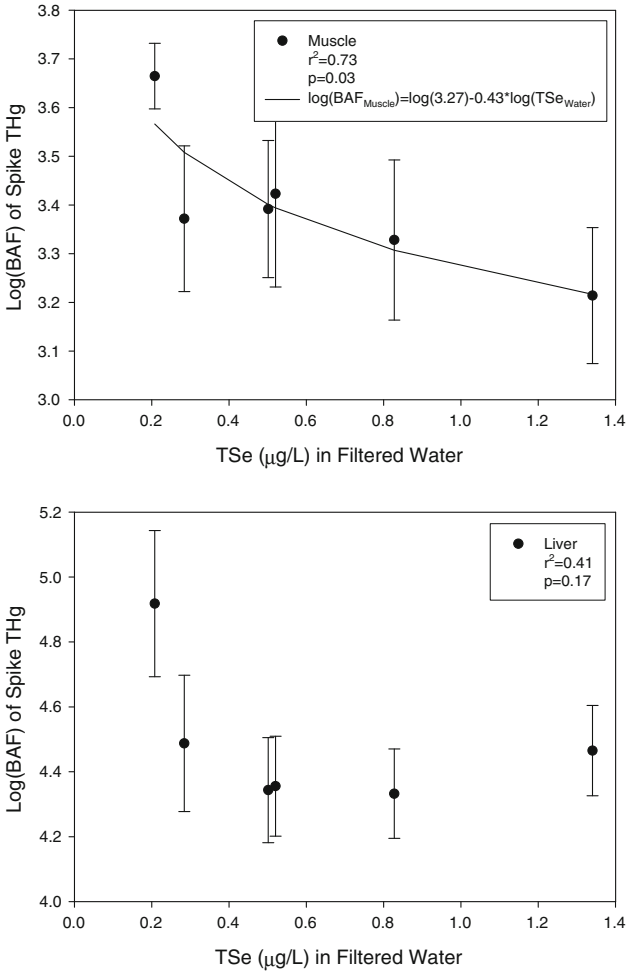

Fig. 5 BAFs of spike THg in muscle of yellow perch as a function of TSe concentrations in filtered water. *Each data point* represents the mean ± 1 SD

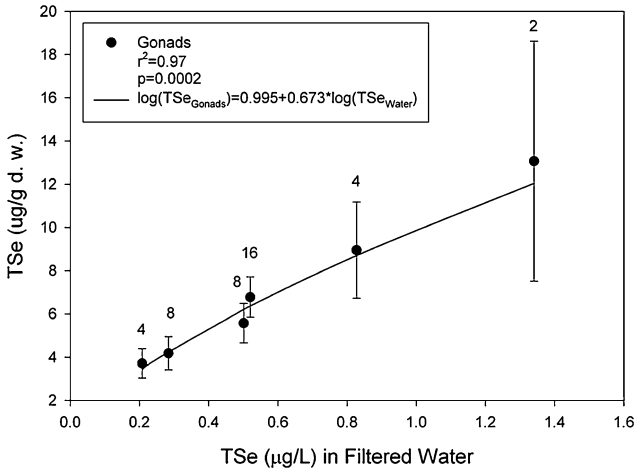

Fig. 6 Concentrations of TSe in gonads of *yellow* perch as a function of TSe concentrations in filtered water. Each data point represents the mean of all measured concentrations ±1 SD. The suggested Se toxicity threshold for gonads is 10 µg/g (dw), whereas the United States Environmental Protection Agency threshold for Se in tissue of fish is 7.9 and 5.8 before winter. The sample size is depicted above the error bars

compared with controls. Addition of Se decreased spike Hg concentrations in muscle of fish by the following amounts relative to the control as calculated from the regression equation: 54 % decrease at 1 µg/L, 49 % decrease at 0.8 µg/L, 41 % decrease at 0.4 µg/L, and 28 % decrease at 0.2 µg/L. It is important to note, however, that mesocosms were not at steady state and that fish were exposed to Se for only 8 weeks.

In a previous study where stable isotopes of spike Hg were added to similar mesocosms, approximately half of the stocked yellow perch contained spike THg after 5 weeks, and almost all contained spike THg after 10 weeks (Orihel 2005). A different mesocosm study found that 15 % of newly acquired Hg was spike Hg after 1 year and that steady state regarding spike Hg was not achieved in fish after two summers of exposure (Paterson et al. 2006).

The results of our study showing decreased Hg concentrations in muscle of yellow perch with the presence of Se in water agree with other studies (Rudd et al. 1980; Turner and Swick 1983; Paulsson and Lundbergh 1989), even though our study used Se concentrations that were lower than in previous studies. In a mesocosm study in Clay Lake, Ontario, Se was added to achieve concentrations of 1, 10, and 100 µg/L of Se. The spike Hg body burden in yellow perch ($n = 3$) was 20 % lower with 1 µg/L of Se (Turner and Rudd 1983). After 2 years of adding Se and lime to whole lakes in Sweden, 60 % less Hg was observed in muscle of yellow perch at 1–2 µg/L of Se, and 85 % less Hg was present at 3–5 µg/L of Se after 2 years (Paulsson and Lundbergh 1991).

Spike Hg Methylation and Bioaccumulation

Decreases in Hg concentrations in muscle of fish with Se additions can arise from several different mechanisms. For example, Se additions could affect Hg methylation (Chen et al. 2001) and/or bioaccumulation (Turner and Rudd 1983; Turner and Swick 1983). In our study, very little of the spike Hg was methylated and returned to the water column (Mailman 2008), yet 65 % of the spike THg in fish was spike MeHg. Most of the MeHg in filtered water was ambient MeHg, whereas most of the THg was spike THg. There was no detectable effect on MeHg concentrations in filtered water or particulate matter related to Se concentrations in filtered water (Mailman 2008); therefore, interactions between Hg and Se related to methylation were not of great importance during the 8 weeks of exposure.

In muscle of yellow perch, approximately 65 % of spike THg was present as spike MeHg before steady-state

conditions were achieved. This is evidence for spike MeHg bioaccumulation by yellow perch. In another mesocosm study (Hecky et al. 1991) and a whole-lake study (Van Walleghem et al. 2007), 85 and 100 % of spike THg that was assimilated into muscle was methylated. The presence of spike MeHg in all biota shows that spike Hg was methylated and bioaccumulated in these mesocosms. The results of this study and the literature indicate that spike Hg is methylated, taken up by the lower food web, and then incorporated into tissues of fish. Although little spike Hg was methylated, this experiment was long enough for spike MeHg to bioaccumulate.

BAFs of MeHg for zooplankton and *Chironomid* larvae were decreased in the presence of increased Se. Because there was no detectable affect on MeHg concentrations in abiotic compartments, the relationship of these BAFs with TSe in filtered water could have been due to differences in the uptake of MeHg from water. The significant negative relationship between the BAF of THg in muscle of fish and Se concentrations in filtered water suggests that the mechanisms by which Se additions decreased Hg concentrations in the food web were likely modifications of Hg uptake or retention. Significant relationships between Hg concentrations in muscle of yellow perch with Se concentrations in filtered water support modification of Hg uptake or retention as the most important modes of action.

A critical finding of this study was the difference in how tissues accumulated ambient and spike Hg. Greater concentrations of spike THg in liver than muscle may indicate that spike Hg was first accumulated in the liver after assimilation. Spike THg apparently first entered the liver and was then transferred to muscle as has been shown in other studies (Van Walleghem et al. 2007; Leaner and Mason 2004). In this study, it appears that Se decreased the amount of spike THg accumulated in liver and thus decreased concentrations of spike THg in muscle of yellow perch, therefore supporting that modification of Hg uptake from food or uptake by fish tissues could explain our observations of decreased Hg concentrations.

Se Concentrations Increased in Gonads of Fish

Rapid bioaccumulation of Se by yellow perch resulted in increased Se concentrations in gonads of fish in only 8 weeks. Presumably, fish exposed to increased Se concentrations for >8 weeks could accumulate even greater Se concentrations in gonads. The main concern for Se toxicity is toxicity occurring during development of fish larvae (Lemly 1998),and increases in Se concentrations in gonads increase the risk of Se toxicity. Se is transferred during egg development from the liver to gonads (Janz et al. 2010). Fish larvae acquire Se from the yolk sack, at which time Se replaces sulphur during protein syntheses, thus causing deformities. A recent review of Se toxicity in fish suggests that concentrations in ovaries of 17–24 μg/g dw are associated with toxicity (Janz et al. 2010). Turnover rates of Se in gonads are not known; however, it is assumed that concentrations would have increased during the remainder of the summer and autumn due to bioaccumulation and also during the winter due to bioconcentration between late summer and the spring spawning period.

Evaluation of Se Addition as a Strategy to Decrease Hg Concentrations in Fish

Low-level addition of Se decreased ambient MeHg concentrations in liver and spike Hg concentrations in muscle. Se applications to selectively treat hydroelectric reservoirs and water bodies affected by point sources of contamination would probably decrease Hg concentrations in fish. The cost of Se addition is relatively low, and application by way of floatplane, boat, in suspended biodegradable rubber, or at an upstream dam would be relatively inexpensive.

Despite these advantages, there are substantial disadvantages to using Se to decrease Hg concentrations in fish. Repeated additions may be required. Se additions would likely affect downstream environments; these effects must be monitored closely; and the effort and cost of monitoring Se concentrations throughout the target and downstream systems could be substantial. There are risks of managing Se to prevent toxicity because the difference between essential levels of Se and levels that may cause toxicity is small (Lemly 1998). Seasonal variations of Se concentrations in water occur (Mailman 2008) and could cause an underestimate or overestimate of the addition amount and either fail to affect Hg or increase the risk of a toxic effect. In addition, winter stress syndrome increases Se concentrations, whereas Se burdens remain similar in fish during the winter (Lemly 1996) causing greater Se concentrations when toxicity is most critical, i.e., before or during vitellogenesis. Adding one substance that could cause negative impacts to treat another could set an important precedent that would better be avoided. Although Se in fish gonads in our experiment did not reach toxic levels, our experiment was of relatively short duration, and it seems likely that toxic levels would have been reached by autumn or the next spring. Concerns about an increased risk of Se toxicity lead us to conclude that addition of Se to lakes to decrease Hg concentrations in muscle of fish should be treated with caution.

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