ASSESSMENT OF MERCURY AND SELENIUM INTERACTIONS IN FRESHWATER

by

MARIAH MAILMAN

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University of Manitoba
Winnipeg, Manitoba
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Of

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ABSTRACT

Additions of selenium (Se) have been shown to effectively lower mercury (Hg) concentrations in the muscle of fish in freshwater in some studies, but the responses of Hg to Se are highly variable, site specific, and concentration dependent. At low concentrations, Se is a nutrient, but at slightly elevated concentrations it can impair development of fish larvae, which can result in failure to recruit. Therefore, the effectiveness of using Se additions to lower Hg concentrations in fish hinges on its ability to do so at low concentrations. Se concentrations may become depressed in newly flooded reservoirs where higher rates of methylation can produce volatile forms of Se. Reservoirs are known to have elevated concentrations of Hg in fish to which depressed Se concentrations could contribute.

We surveyed reservoirs and lakes in Québec and Ontario to assess natural background concentrations of Se and evaluate whether flooding caused depression of Se concentrations. We also ran a mesocosm experiment based on a regression design to evaluate the effectiveness of low Se concentrations to lower Hg concentrations in yellow perch and the lower food web without imposing toxicity.

In the reconnaissance survey, concentrations of Se in fish, zooplankton, emerging insects, sediment, and water were not consistently depressed in reservoirs relative to reference lakes. Concentrations of Hg and Se in muscle of fish correlated inversely from the ELA and positively in fish from La Grande Complex, Québec. The prey of fish met or exceeded dietary requirements of Se in the diet of fish, even in systems with low Se concentrations.

Results from the mesocosm experiment indicate concentrations of spike Hg in muscle and liver of fish inversely correlated with Se concentrations in water after eight weeks of exposure. About 65% of the spike THg in muscle occurred as spike MeHg. Presumably Hg concentrations in muscle of fish would become lower with longer exposure. Increasing concentrations of Se in water from about 0.2 to 1.0 µg/L resulted in Hg concentrations in muscle of fish that were 54% lower relative to controls. The most conservative toxicity threshold for Se in tissue of fish is 7.9 µg/g (dry weight). At 0.7 µg/L of Se in water for eight weeks, gonads of fish contained about 8 µg/g (dry weight) of Se. This could increase with longer exposure. Thus, Se concentrations in water should be well below 0.7 µg/L to prevent the possibility of Se toxicity. Also, there was a significant relationship between Se concentrations in muscle and gonads of yellow perch, which could enable non-lethal monitoring of this species for Se concentrations in gonads. Additions of Se did not affect partitioning of Hg in the physical-chemical environment, with the exception that concentrations of ambient MeHg in water were

positively related to Se concentrations in water. In addition, the amount of Se present increased the partitioning of Se^{4+} to particulate matter while it decreased the partitioning of Se^{6+} to this compartment. Evasion of Hg to the atmosphere was not measured, but we assume this to be the largest sink for spike Hg followed by sediment, water, and then particulate matter. While Se additions lowered Hg concentrations in yellow perch, it also elevated Se concentrations in gonads and thus we advise not adding Se to aquatic systems because of the risk of Se toxicity. Se addition is not advised as it would be difficult to maintain target Se concentrations in light of seasonal variability, winter stress in fish, accumulation in downstream habitats, and it may set an important precedent.

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GENERAL INTRODUCTION

The purpose of this research is to assess the interactions between mercury (Hg) and selenium (Se) in freshwater environments towards lowering Hg concentrations in fish. This is an important topic to the scientific community and environmental managers because Hg is a world-wide contaminant and many systems are in need of remediation, and because Se is a potential toxin at elevated concentrations in the environment. Our work assesses the lowest possible concentrations of Se that could effectively lower Hg concentrations in the tissues of fish and the risk of Se toxicity to fish using a range of scientific and experimental approaches. It also examines the partitioning and fate of Hg at different doses of Se. The theories on how Se interacts with Hg to reduce its uptake are by lowering Hg methylation (Chen *et al.* 1997; Jackson 1991; Jin *et al.* 1999), modifying Hg transfer through the food web or uptake by fish (Turner and Rudd 1983; Turner and Swick 1983), and increasing loss of Hg from fish tissues (Bjerregaard *et al.* 1999; Pedersen *et al.* 1998).

Cycling of Hg

There is thought to be a direct link between the atmospheric deposition of inorganic mercury (Hg) and methyl mercury (MeHg) in freshwater food webs (Munthe *et al.* 2007). Natural processes redistribute Hg, such as weathering of rocks, volatilization from soil and water, volcanic out-gassing, and wildfires. However, human activities have altered the biogeochemical cycle of mercury (Hg) throughout the industrial era. Combustion of fossil fuels and combustion of waste from industries, municipalities, and medical institutions have increased the amount of Hg in the biosphere by two to three times (Mason *et al.* 1994).

Hg cycles throughout the atmosphere, terrestrial landscapes, aquatic environments, plants, and animals. After being deposited from the atmosphere, Hg becomes associated with, and is retained by, organic carbon (Fitzgerald and Lamborg 2004), and it accumulates in vegetation and soil. In carbon-rich and oxygen-poor environments, microorganisms convert inorganic Hg to the relatively more toxic form MeHg (Compeau and Bartha 1985). In lakes, zooplankton may take up MeHg from water (Monson and Brezonik 1998; Peech Cherewyk 2002) and by ingesting suspended particulate matter or phytoplankton (Mason *et al.* 1996; Plourde *et al.* 1997). Benthic invertebrates acquire MeHg from detritus or filter feeding (Hall *et al.* 1998). Fish mainly acquire MeHg through dietary exposure (Hall *et al.* 1997; Bodaly and Fudge 1999). Thus, MeHg transfers easily among trophic levels (Back and Watras 1995; Hill *et al.* 1996) and it biomagnifies up the food chain (Watras and Bloom 1992; Cabana *et al.* 1994;

Tremblay *et al.* 1996; Chen *et al.* 2000). The predominant form of Hg in fish is monomethyl Hg (MeHg; Bloom 1992). The main source of Hg to humans and wildlife is MeHg in fish.

Fish are often eaten or sold as food. Consumption of fish is the most important route of Hg exposure to humans (Richardson *et al.* 1995; Mergler *et al.* 2007). In vertebrates, MeHg affects the central nervous system ranging from subtle learning difficulties to death (Mergler *et al.* 2007). Lowering Hg in fish would have health benefits for humans and lowering Hg emissions could lead to lower Hg concentrations in fish.

Anthropogenic and natural emissions of Hg eventually fall to the surface of the earth where they bind to carbon. Flooding terrestrial landscapes increases rates of Hg methylation (Hecky *et al.* 1991; Kelly *et al.* 1997; Heyes *et al.* 2000; Bodaly *et al.* 2004). After flooding, organic carbon decomposes and may release carbon, deplete the water of oxygen, and remobilize Hg. Under these conditions, sulphate reducing bacteria (SRB) can convert inorganic Hg to MeHg (Gilmour *et al.* 1992; Choi and Bartha 1994). Further, methylation of Hg may be greatest in organic-rich sediment (Compeau and Bartha 1985; Choi and Bartha 1994; Pak and Bartha 1998; Macalady *et al.* 2000), such as flooded soils. For example, production of MeHg increased within months of flooding hydroelectric reservoirs in northern Québec (Plourde *et al.* 1997) and experimental reservoirs in northwestern Ontario (Hall *et al.* 2005). Uptake of MeHg and its transfer through the lower food web increases as a result of flooding (Tremblay and Lucotte 1997; Tremblay *et al.* 1998; Paterson *et al.* 1998; Hall et al. 1998). Flooding terrestrial landscapes further elevates Hg concentrations in fish (Lodenius *et al.* 1983; Bodaly *et al.* 1984; Verdon *et al.* 1991; Bodaly and Fudge 1999; Hall *et al.* 2005). In recent years some hydroelectric reservoir developments have limited the amount of Hg in fish by flooding less area and therefore causing less stimulation of Hg methylation. This has been done in steep-sided river valleys; however, other strategies are required in areas of flat topology.

One possible mitigation approach to reduce Hg in fish is to add small amounts of selenium (Se) to aquatic ecosystems. It is unknown if adding Se can lower Hg in fish without increasing the risk of Se toxicity. Selenium easily transfers from female fish to their eggs and is then acquired by larvae from their yolk (Lemly 1998). Relatively low concentrations of Se can cause deformities to offspring or failure to recruit, without any signs of toxicity in adult fish. This occurs because excess Se can replace sulphur during protein synthesis, alter the structure of proteins, and alter the function of proteins (Lemly 1998).

The effectiveness of Se in lowering Hg was experimentally evaluated in the 1980s by two independent research groups. In Canada, mesocosm studies demonstrated the effectiveness

of Se at lowering Hg in yellow perch, white sucker, and northern pike (Turner and Rudd 1983). Whole-lake studies in Sweden further established that Hg decreased in fish when Se was added (Paulsson and Lundbergh 1989; Paulsson and Lundbergh 1991). These countries responded differently to this information. Sweden continued to add low levels of Se to manage Hg contaminated fish for human consumption. Canada refrained from using Se for mitigating Hg contamination because of potential Se toxicity to fish. Instead, other management methods have been employed to lessen Hg exposure to people. These include fish consumption advisories, providing frozen fish to some First Nations communities, closing coal-fired power plants, building "run-of-the-river" reservoirs instead of flooding large areas, researching clean-coal technology, and implementing educational and rebate programs to promote energy conservation. After Hg emissions drop, long lag-times for corresponding decreases in Hg concentrations in fish may require ongoing management. Although many possibilities have been considered, no large-scale, economically feasible strategies are in use. We re-evaluated Se addition as a possible Hg management strategy including the potential for Se toxicity to fish.

Cycling of Se

Selenium occurs in all environmental compartments, but it occurs at higher concentrations in certain environments such as uranium deposits, mineralized veins, phosphoric mining, and hot springs. It is emitted to the environment from human activities such as in landfill gases and smelting of copper and nickel ores (Chen *et al.* 2001). Human uses of Se include manufacturing of ceramics, glass, and vulcanized rubber, as an agricultural fungicide, in medical shampoo, in photocopy machines and televisions, and to facilitate structural analysis of protein by x-ray diffraction. Effluents from coal-fired power plants are enriched in Se. Atmospheric deposition from burning coal is an important source of Se to terrestrial and aquatic environments (Lawson and Mason 2001).

Selenium may exist in oxidized and reduced forms. Reduced forms of Se often bind with carbon. Inorganic forms of Se include selenite (Se^{+6}; SeO_3^{2-}), selenate (Se^{+4}; SeO_4^{2-}), and elemental Se (Se^0). Organic selenides (Se^{-2}) occur in many selenioamino acids such as selenomethionine and selenocysteine (reviewed in Cutter and Cutter 1998). Reduction of oxic anions to elemental Se occurs at the oxic-anoxic interface in aquatic systems (Cutter 1992). Formation of volatile Se, predominantly found in natural waters as dimethyl selenide (DMSe), leads to atmospheric emissions and distribution.

In aquatic systems, Se may be chemically transformed, deposited, redistributed, bioconcentrated, and bioaccumulated. Selenium can be present as selenite, selenate, or diselenite in aerobic sediments (Masscheleyn *et al.* 1991) and as elemental Se or metal selenides (ie. FeSe) in anoxic settings (Masscheleyn and Patrick 1993; Peters *et al.* 1997). Elemental Se converts quickly to selenite on oxidation (Masscheleyn *et al.* 1990). Bioturbation causes acceleration of pore water exchange, physical mixing, and localized oxidation of sediments, and this may remobilize Se from sediments to the overlying water (reviewed in Peters *et al.* 1999a).

Selenium can be methylated both abiotically and by microorganisms. Organic Se compounds, such as selenomethionine, can change to volatile Se forms in light and dark settings by abiotic mechanisms, cleavage or hydrolysis. Dimethyl selenide (DMSe) is the main volatile species found in water. Because DMSe constitutes only a small fraction of the products of abiotic methylation of organic Se, it may be formed as the breakdown product dimethyl diselenide (Amouroux *et al.* 2000).

Selenium is an essential element for eukaryotes and bacteria (Guimaraes *et al.* 1996). Bacteria accumulate selenite and selenate. Microbial metabolites of Se are DMSe (Chasteen 1993), dimethyl diselenide (Chasteen 1993; Peters *et al.* 1999b), and dimethyl selenenyl sulphide (Chasteen 1993; Fan *et al.* 1997; Amouroux and Donard 1997; Fan and Higashi 1998; Van Fleet-Stalder *et al.* 2000). In addition, methanelelenol (CH_3SeH) was identified in microorganisms from reservoirs (reviewed in Chasteen and Bentley 2003). Chasteen and Bentley (2003) have extensively reviewed the mechanisms for Se biomethylation.

Algal uptake of Se varies with cell volume suggesting enzyme mediated uptake rather than simple adsorption. At water concentrations of 3 to 10 µg/L of Se, selenite accumulation by algae increased at lower pH levels (Riedel and Sanders 1996) suggesting selenoamino acids could accumulate in algae more readily than charged species (Besser *et al.* 1993; Baines *et al.* 2001). In freshwater, uptake of Se(IV) by algae is proportional to irradiance and primary production (Baines *et al.* 2004). Community composition can greatly influence the concentration of Se in phytoplankton, and therefore the transfer of Se through aquatic food webs (Baines and Fisher 2001). Also, bacteria concentrate selenite, mainly as selenoamino acids, at concentrations up to 13 times greater than those in phytoplankton (Baines *et al.* 2004). Diet is the primary rout of exposure of Se to zooplankton and they concentrate selenite more readily than selenate, but selenomethionine, an organic form of Se, is the most easily accumulated species of Se (Besser *et al.* 1993) and its production is essential for Se bioaccumulation.

Benthic invertebrates bioaccumulate Se from sediments, diatoms, bacteria, and water (Luoma *et al.* 1992). Exposure of benthic invertebrates to Se is thought to be primarily from organic detritus in sediments rather than from waterborne Se (Canton and Van Derveer 1997; Peters *et al.* 1999a), and their bioturbation may remobilize Se from sediment into the food web (Peters *et al.* 1999b). Selenium in benthic invertebrates was associated with proteins and partially characterized as selenomethionine (Peters *et al.* 1999b). Selenium concentrations can decrease with increasing trophic level in food webs (Besser *et al.* 1993; Mason *et al.* 2000); however, bioaccumulation of Se is specific to the food web pathway (Stewart *et al.* 2004) and it is the benthic food web that seems to contribute the most to Se biomagnification (Baines *et al.* 2004; Stewart *et al.* 2004). The main source of Se to fish is through dietary exposure (Turner and Swick 1983; Baines *et al.* 2004).

Objectives

We created a conceptual model to explain the steps within the Hg cycle at which Se can have an effect (Table 1). The effects we focused on were modification of Hg methylation and bioaccumulation. This project involved two separate studies. The first part was a reconnaissance survey to evaluate concentrations of Se in hydroelectric reservoirs and lakes in Québec. Before adding Se it is necessary to know how much Se is already present to determine how close background concentrations are to toxicity thresholds. Hg concentrations are typically high in fish from environments that are high in organic matter and low in oxygen, such as new reservoirs. Under these conditions, Se may also be lowered by losses resulting from the formation of volatile Se species. In new reservoirs, Hg concentrations in fish are known to be elevated. If Se lowers Hg in fish, the absence of Se may contribute to elevated Hg concentations in fish. In this hypothesis, additions of Se to reservoirs would restore Se and may be more acceptable than additions beyond what is deemed required or natural. The second phase of our approach was a mesocosm study. We evaluated the effectiveness of low concentrations of Se to lower Hg bioaccumulation and associated risks of Se toxicity. Finding the lowest effective Se concentration prevents excessive Se additions and potential Se toxicity. Our research builds on past studies by lowering the range of Se concentrations tested, 0.1 to 1.6 μg/L of Se with no Se added to the controls. Past studies only assessed acute Se toxicity. Assessing chronic toxicity, which is currently known to manifest through elevated Se concentrations in gonads and to affect reproduction, gives resource managers more information with which to make decisions about Se additions.

In summary the null hypotheses were:

1. there is no effect on concentrations of Se in environmental compartments of reservoirs,
2. changes in the concentrations of Se caused by flooding do not affect Hg concentrations in environmental compartments,
3. Hg partitioning among environmental compartments is not related to Se concentrations in water,
4. MeHg concentrations in environmental compartments are not related to Se concentrations in filtered water,
5. there is no effect of Se on Hg concentrations in fish,
6. concentrations of Se in gonads of fish will be below the toxicity threshold of 10 μg/g after Se additions.

In hypotheses 3 to 6 we manipulated Se concentrations in mesocosms, knowing it would accumulate through the food web and possibly cause Se toxicity. We also manipulated Hg concentrations using stable isotopes for the purpose of tracing the transfer of Hg through the food web to assess the impacts of adding different Se concentrations. We expected our highest Se treatment (1.6 μg/L) to have lower Hg concentrations in fish because in past studies 1 μg/L of Se lowered Hg concentrations in fish. We were uncertain if we would observe elevated Se concentrations in gonads of fish because our water concentrations were lower than those that caused developmental deformities in Belews Lake (2 μg/L; Lemly 2002).

Each chapter of this thesis will be submitted for publication to the following journals:

1. A review of mercury and selenium interactions in freshwater fish toward lowering mercury in fish will be submitted to Science of the Total Environment,
2. Are concentrations of selenium lower in reservoirs and does selenium affect mercury cycling? Results from a reconnaissance survey will be submitted to Environmental Pollution,
3. Fates and mass balances of selenium and isotopic mercury added to mesocosms will be submitted to Environmental Science and Technology,
4. Selenium additions lowered mercury concentrations in fish and increased selenium concentrations in gonads will be submitted to Environmental Science and Technology.

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TABLES

Table 1. Conceptual model of the Hg cycle and the possibilities for interactions with Se.

MERCURY CYCLE STEP	EFFECT OF SELENIUM
Deposition of Hg into lakes	
Reduction and volatilization	
Sedimentation →	Form insoluble complex
Methylation of Hg →	Lower methylation of Hg
Reduction and volatilization	
Sedimentation	
Bioconcentration	
Uptake of Hg by lower food web →	Modify uptake of Hg by lower food web
Uptake of Hg by higher food web →	Modify uptake of Hg by higher food web
Loss of Hg from fish →	Increase loss of Hg from fish

A REVIEW OF MERCURY AND SELENIUM INTERACTIONS IN FRESHWATER FISH TOWARD LOWERING MERCURY IN FISH

ABSTRACT

As elevated mercury (Hg) is the main reason for fish consumption advisories in North American freshwaters, it is important to find ways to lower Hg concentrations in fish to those that are lower risk to consumers. Selenium can lower Hg concentrations in fish, but there are real risks of Se toxicity. We review the possibility of adding selenium (Se) to lakes and hydroelectric reservoirs as a strategy to lower Hg in fish from contaminated systems. Mechanisms by which Se lowers Hg concentrations in fish are unclear but could include limiting trophic transfer of Hg between fish and their prey, increasing the loss of Hg from fish, or lowering Hg methylation in lake and reservoir sediments. We summarize and critique a history of case studies and provide a synthesis of the findings. The results of the case studies were assessed for evidence for the following: Se lowers Hg bioaccumulation, Se and Hg concentrations are negatively correlated in muscle of fish, no relationship or a positive correlation between Se and Hg concentrations in muscle of fish in rivers, effects of Se on survival of fish exposed to Hg, loss of Hg from fish, and Se affects rates of Hg methylation in sediment. We also use an example from the literature to estimate what concentration of Se would be needed to lower Hg concentrations in fish to those that meet safe consumption guidelines. Finally we outline evidence of Se toxicity to freshwater fish. There is experimental evidence that Se lowers Hg concentrations in fish by 10 to 50% at 1 to 3 μg/L of Se, but at these concentrations Se toxicity has been observed in other studies. Most studies used unrealistic concentrations, exposure routes, or an inappropriate chemical form of Hg or Se. There have not been consistent results that demonstrate the relative importance of the possible modes of interaction between Hg and Se. The concentrations of Se required to lower Hg in fish would likely impose chronic toxicity of Se to fish.

INTRODUCTION

Fish are a nutritious and a widely available food, but mercury (Hg) contamination from fossil fuel combustion and reservoir construction threaten the health of those who eat fish (Mergler *et al.* 2007). Industries emit Hg that travels long distances through the atmosphere, settles on land and water in dust or dissolved in rain and snow, and then adsorbs to carbonaceous matter (Fitzgerald and Lamborg 2004). Mercury in elemental or ionic form is not a great health concern until it is microbially converted to methyl mercury (MeHg; Compeau and Bartha 1985).

Reservoir construction also results in elevated concentrations of Hg in fish. Damming flowing water to create hydroelectric reservoirs can flood large areas of land and therefore large amounts of organic carbon that sequester Hg. Flooding organic carbon remobilizes Hg and increases MeHg production and causes elevated Hg concentrations in fish (Bodaly *et al.* 1984; Bodaly and Fudge 1999; Hall *et al.* 2005; Hecky *et al.* 1991; Verdon *et al.* 1991).

Methyl Hg is accumulated from water by phytoplankton and zooplankton (Paterson *et al.* 1998; Watras *et al.* 1998) and transferred to organisms through their diet (Bodaly and Fudge 1999; Hall *et al.* 1997; Hall *et al.* 1998). The predominant form of Hg in fish tissue is MeHg (Bloom 1992). Methyl Hg bioaccumulates (Back and Watras 1995; Hill *et al.* 1996) and biomagnifies (Cabana *et al.* 1994; Chen *et al.* 2000; Watras and Bloom 1992) causing organisms that are large, old, and/or preying at high trophic levels to acquire elevated Hg concentrations (reviewed in Bodaly *et al.* 1997). Eating fish is the main source of MeHg to humans and other top predators. Toxic endpoints of MeHg in humans are the nervous system, the reproductive system, the cardiovascular system, and the immune system (Mergler *et al.* 2007).

Persistent Hg contamination in freshwater lakes and hydroelectric reservoirs from non-point sources can be addressed by managing fish consumption, reducing Hg emissions from industries, and remedial action. Consumption advisories for freshwater fish with elevated MeHg concentrations, 0.5 μg/g for commercial sale in Canada (Health Canada 2007), manage the exposure of MeHg to humans but not to wildlife. Most fish consumption advisories are due to elevated Hg concentrations; in the United States, 38% of the lake acres, 26% of river miles, and 65% of coastlines have advisories for fish consumption due to elevated Hg concentrations (USEPA 2007). Avoiding fish in one's diet can disrupt social, cultural, economic, spiritual, physical, and emotional wellness (Niezen 1999; Rosenberg *et al.* 1997). If Hg emissions ceased, widespread lower MeHg concentrations in fish would likely occur (Madsen and Stern 2007; Orihel *et al.* 2006), but the lag time could be great due to large amounts of Hg stored in terrestrial organic material.

In the lag time between reducing Hg emissions and subsequent decreases in Hg concentrations in fish, a remedial strategy, such as adding selenium (Se) to lower fish muscle Hg concentrations, may limit MeHg exposure to humans and wildlife (Paulsson and Lundbergh 1989; Paulsson and Lundbergh 1991; Rudd *et al.* 1980; Turner and Rudd 1983; Turner and Swick 1983). In four studies, Se additions have quickly and efficiently lowered MeHg concentrations in muscle of fish in lakes and reservoirs, but the extent to which it is lowered needs further examination. In addition, Se is highly toxic and the risks of Se toxicity caused by additions need to be assessed further.

A problematic aspect of Se additions is that Se can cause toxicity to fish at low concentrations. Selenium is essential and required in fish diet at 0.1 to 0.5 μg/g (Hodson and Hilton 1983), but at slightly higher concentrations, it can cause toxicity. The recommended toxicity threshold for Se in the diet of fish is 3 μg/g (d.w.; Lemly 2002). Selenium teratogenesis occurs at concentrations exceeding 10 μg/g in gonads of fish (d.w.; Lemly 2002) and the accepted threshold for Se in tissue of fish is 7.91 μg/g (d.w.; USEPA 2006). The proposed mechanisms by which Se induces toxicity are substitution of Se for sulphur during the formation of proteins causing terata, edema, and pathology in the liver, kidney, brain, heart, and eyes (reviewed in Lemly 2002b). In addition, excessive Se can form superoxide that induces oxidative stress in embryos of fish (Palace *et al.* 2004b). These symptoms result in reduced growth and survival, which can result in failure to recruit or elimination of fish species from a system (reviewed in Hamilton 2004; Lemly 2002). Also, riparian wetlands, which are feeding and spawning habitats, accumulate Se. Adding Se could cause Se concentrations to become elevated in riparian wetlands, elevate the exposure of fish and birds to Se, and therefore increase the risk of selenosis (Lemly 1999). Further, seasonal changes such as winter stress syndrome can elevate Se concentrations and toxicity in fish (Lemly 1993; Lemly 1996). Another complication to predicting Se exposure is that the uptake of Se is determined by physiological and ecological processess, such as food web structure and particularly uptake of Se at the base of the food webs (Stewart *et al.* 2004).

Before employing Se additions, the mechanisms by which Se and Hg interact must be understood. The interaction between Se and Hg can result in antagonism, no effect, or synergism. Antagonism is when Se lowers the effects of Hg or lowers Hg bioaccumulation. Synergism is when Se enhances the toxicity or uptake of MeHg. In field experiments, Se addition has lowered Hg concentrations or bioaccumulation (Paulsson and Lundbergh 1989; Paulsson and Lundbergh 1991; Rudd *et al.* 1980; Turner and Rudd 1983; Turner and Swick

1983). Concentrations of Hg and Se in the muscle of fish have correlated negatively in lakes (Chen *et al.* 2001; Leskinen *et al.* 1986; Mauk and Brown 2001; Southworth *et al.* 2000; Southworth *et al.* 1994; Speyer 1980), positively for other fish species in rivers (Burger *et al.* 2001; Dorea *et al.* 1998; Lima *et al.* 2005), or not at all in lakes and rivers in the same studies. In fish exposed to Hg, Se may extend survival (Cuvin and Furness 1988; Siegel *et al.* 1991), but decrease hatchability (Huckabee and Griffith 1974). Selenium may also redistribute or increase loss of Hg from fish (Bjerregaard *et al.* 1999; Pedersen *et al.* 1998). In addition, the interaction among Se and Hg may be external to the fish. For example, Se may lower the rate of Hg methylation in sediment of aquatic environments (Chen *et al.* 1997; Jackson 1991; Jin *et al.* 1999). Studies researching the cellular interactions of Se and Hg in fish (Baatrup and Danscher 1987; Suzuki *et al.* 1998) are not discussed.

Additions of Se affect Hg uptake in the food chain but there is a real possibility of Se toxicity. Below, for each mechanism we synthesize the findings and critique the merit of the results from these studies. Our objective is to describe Se and Hg interactions in freshwater fish with regard to Se addition to lower MeHg in fish and assess whether this strategy could be employed without causing toxic effects.

MECHANISMS

Experimental demonstration of Se lowering Hg bioaccumulation in fish

Sodium selenite additions have lowered MeHg concentrations in fish in microcosm, mesocosm, and whole lake experiments. This may occur due to Se and Hg interactions in sediment, by modifying uptake or trophic transfer, or due to physiological modification. Regardless of the mechanism, Se additions have lowered Hg concentrations in fish in various settings.

Case Studies

In Clay Lake, Ontario, Rudd *et al.* (1980) added 100 µg/L of Se, plus 2 mCi of ^{75}Se to a mesocosm and compared ^{203}Hg bioaccumulation in the presence of Se to a control mesocosm to which no Se was added. After two weeks, pearl dace (*Semotilus margarita*) accumulated Se rapidly (0.1 µg/g per day). In the Se treatment, the rate of uptake of spike Hg into muscle of pearl dace, muscle of crayfish, and benthic invertebrates was half of those in the control.

Turner and Rudd (1983) also studied the bioaccumulation of Se and Hg using radioactive isotopes added to five 10-m diameter mesocosms in Clay Lake, Ontario, for six weeks.

Additions of 1 μg/L of sodium selenite lowered the ^{203}Hg body burden in yellow perch (*Perca flavescenes*; n=3) and juvenile white sucker (*Catostomus commersoni*; n=6) by about 20% (Turner and Rudd 1983). Additions of 10 μg/L of sodium selenite lowered the ^{203}Hg body burden in yellow perch (n=3) by 30% and juvenile white sucker (n=6) by 50%. Additions of 100 μg/L of sodium selenite lowered the ^{203}Hg body burden in yellow perch (n=3) by 50% and juvenile white sucker (n=1) by 40%. Bioaccumulation of ^{203}Hg was not lowered in clams, snails, or zooplankton (Turner and Rudd 1983). Selenium was rapidly taken up by biota. Turner and Rudd (1983) only considered lethal effects of Se to adult fish and they found no behavioural changes or mortalities from Se exposure when whole bodies of fish contained 0.5 to 5.0 μg/g (w.w.) of Se.

In northern pike (*Esox lucius*) in mesocosms in Clay Lake, Ontario, dietary exposure of Se was responsible for lowering Hg assimilation (Turner and Swick 1983). Selenium in water and food lowered assimilation of ^{203}Hg in northern pike whole body ($p<0.03$) and muscle ($p=0.001$) by 30 to 60%.

Turner and Swick (1983) suggest that the experiment be run again for a longer time to examine the assimilation of ^{203}Hg when fish are exposed to Se. They also suggest determining the lowest Se to Hg ratio that effectively lowers Hg assimilation. In addition, we suggest the rate of Hg loss from fish could be evaluated by pre-exposing fish to a different stable isotope of Hg, and chronic Se toxicity should be assessed at these concentrations.

Klaverkamp *et al.* (1983a) conducted two experiments to establish whether waterborne sodium selenite had direct effects on Hg concentrations in northern pike (*Esox lucius*) and to evaluate the effects of waterborne sodium selenite pre-treatment on the bioconcentration of ^{75}Se, ^{203}Hg, and ^{203}MeHg from water. After 12 days, 1 μg/L of Se significantly lowered Hg concentrations in muscle of fish, while 100 μg/L of Se significantly increased Hg concentrations ($p<0.05$). In the second experiment, northern pike were distributed in microcosms for seven days at 0, 1, 10, and 100 μg/L. The uptake of MeHg was less at lower Se concentrations whereas MeHg bioaccumulation was maintained at high Se concentrations.

Selenium addition in Lake Öltertjärn, Sweden, according to Paulsson and Lundbergh (1989), lowered Hg concentrations in three fish species. Se concentrations in lake water were increased from 0.4 to about 5.0 μg/L for three years. Sodium selenite addition lowered Hg concentrations in muscle of yellow perch (*Perca flavescens*), roach (*Rutilus rutilus*), and northern pike (*Esox lucius*). Mean Hg concentrations after three years were lowered by 84% in yellow perch and 90% in northern pike and roach.

Selenium increased in roach muscle from 0.3 to 0.5 μg/g before Se addition to 11 to 12 μg/g three years later (Paulsson and Lundbergh 1989). Selenium concentrations in muscle of yellow perch increased from 0.5 to 15 μg/g, and in muscle of northern pike it increased from 0.5 to 4 μg/g. In liver of yellow perch, Se concentrations increased from 0.4 to 3.3 μg/g before Se addition to 9.6 to 27 μg/g and in liver of northern pike from 7.8 to 9.4 μg/g before Se addition to 20 to 38 μg/g.

Muscle of yellow perch contained Se concentrations that exceeded the toxicity threshold (Paulsson and Lundbergh 1989). The toxicity threshold for Se in muscle of fish is 7.91 μg/g (d.w.), and when sensitive fish species will be over-wintering, their concentrations before winter should not exceed 5.85 μg/g (d.w.; USEPA 2006). Paulsson and Lundbergh (1991) added sodium selenite to 11 lakes in Sweden that were contaminated with Hg and were also receiving lime treatment. The water concentrations of Se were raised to 1 to 2 μg/L for three lakes and to 3 to 5 μg/L in eight lakes for two years beginning in 1984. In neutral water that had been treated with lime, 2 μg/L of sodium selenite effectively lowered Hg burdens in muscle of fish. The addition of lime lowered Hg burdens in fish by 30% relative to those reference lakes. When Se was added to lakes, lime was also added. Therefore, the percent decrease in Hg after Se addition includes the effect of adding lime. This makes it difficult to separate the effect of Se.

Paulsson and Lundbergh (1991) report 60% lower mean Hg concentrations in muscle of yellow perch in lakes with 1 to 2 μg/L of Se and 85% lower mean Hg concentrations in lakes with 3 to 5 μg/L of Se. In eight lakes, northern pike that had Hg concentrations in muscle at or above 1 μg/g decreased by 22% after one year and by 28% after two years. Northern pike that contained the greatest Hg concentrations before Se addition exhibited the greatest reductions in Hg concentrations compared to those with lower ambient Hg concentrations.

Selenium concentrations increased in the muscle of all fish species, but did not correlate to the dose or the aqueous Se concentration (Paulsson and Lundbergh 1991). Northern pike contained lower Se concentrations than yellow perch.

Synthesis

Many of the studies presented in this section used unrealistically high concentrations of Se. In all cases, the concentrations of Se used were near or exceeded concentrations that have caused toxic effects to fish progeny. It is likely these authors were unaware of the high sensitivity of fish progeny to slightly elevated concentrations of Se. The lowest and most realistic concentration of sodium selenite added was 1 μg/L. Ideally Se concentrations in water

should be limited to 0.5 µg/L (V. Palace, pers. comm.). Selenium toxicity is better understood now compared to when these studies were conducted.

Turner and Rudd (1983) suggested that fish at higher trophic levels may need less Se to facilitate a decrease in Hg concentrations. In 11 lakes, the percent reduction of Hg in muscle of northern pike was less than in yellow perch (Paulsson and Lundbergh 1991). The results of Paulsson and Lundbergh (1991) indicate that the percent reduction in Hg in muscle is greater in fish with lower trophic status after two years of sodium selenite and lime addition. It is reasonable to assume that larger fish with more Hg will take longer to lose Hg than smaller or younger fish with less Hg. Rudd and Turner (1983) propose that because Se bioaccumulates, maintaining elevated concentrations of Se would not be necessary; however, higher trophic level fish did not bioaccumulate as much Se as fish at lower trophic levels (Paulsson and Lundbergh 1991) and therefore the duration of Se application may need to be extended, depending on the mode of interaction. It may take longer for Hg concentrations to decrease in large fish compared to small fish. Therefore it seems Se would need to be elevated for more than a few years in lakes with elevated Hg concentrations to generate a significant loss in Hg in older and larger fish.

Concentrations of Hg and Se correlate negatively in muscle of fish in lakes

Negative correlations of Hg and Se can suggest an antagonistic mechanism by which lower Hg concentrations occur at higher Se concentrations. This relationship was first reported for freshwater fish from Québec (Speyer 1980). Subsequently, significant negative correlations were observed between Se and Hg concentrations in muscle of fish in Finland (Leskinen *et al.* 1986), in Lake Oahe, South Dakota (Mauk and Brown 2001), and in Sudbury, Ontario (Chen *et al.* 2001), but when the Sudbury lakes were re-sampled, Belzile *et al.* (2006) found no correlation. Southworth *et al.* (1994; 2000) had a unique opportunity to monitor Hg concentrations in muscle of fish as Se concentrations in water were lowered. Concentrations of Hg in muscle of fish increased despite elevated Se concentrations in muscle when Se concentrations in water decreased. In contrast, there was no relationship between Se and Hg concentrations in muscle of fish in lakes in Norway (Froslie *et al.* 1985).

Many of the studies draw conclusions that could be supported with bioaccumulation factors (BAFs). Calculating the BAFs of MeHg in muscle of fish indicates how much MeHg is being transferred from water or food to fish. The BAF is the concentration of Hg in tissue divided by the concentration of Hg in water or food. Correlating BAFs with Se concentrations

in water or food will indicate whether exposure to Se is affecting the amount of MeHg bioaccumulation.

Case Studies

Froslie *et al.* (1985) reported Se and Hg concentrations in muscle of fish from lakes in Norway. There were positive correlations between Hg and Se concentrations in muscle of yellow perch from lakes Mjosa ($n=52$; $r^2=0.26$) and Losna ($n=20$; $r^2=0.62$). An inverse correlation was observed in Lake Ottsjoen ($n=20$; $r=0.18$). Correlations of Hg and Se concentrations in muscle of yellow perch from all areas were near zero. Froslie *et al.* (1985) ruled out any significance in these correlations due to small sample sizes, and as a result, we assume there was no significant relationship between Hg and Se concentrations in muscle of yellow perch.

Leskinen *et al.* (1986) evaluated the relationship between Se and Hg concentrations in northern pike (Esox lucius) in Finland in natural lakes and reservoirs. There was a negative correlation between Hg and Se concentrations in muscle of northern pike from both reservoirs and natural lakes ($n=105$; $r^2=0.20$; $p<0.001$), but not for individual lakes. In reservoirs, fish contained lower Se concentrations and higher Hg concentrations than natural lakes, but Se concentrations did not explain Hg content in individual lakes. Because water chemistry did not differ significantly among reservoirs and natural lakes, Leskinen *et al.* (1986) suggested the higher Se concentrations in lakes lowered the proportion of Hg in muscle

In liver of northern pike, high concentrations of Se corresponded to lower MeHg concentrations (Leskinen *et al.* 1986). The percentage of the total Hg that occurred as MeHg was lowest (74%) in fish that had the highest Se concentrations in liver. The presence of Se in liver seemed to decrease the proportion of total Hg that occurred as MeHg in the liver. Because the lowest percentage of MeHg was found in liver that contained the highest Se concentrations, Leskinen *et al.* (1986) speculated that Se and Hg interact in liver of fish.

From 1967 to 1989, Rogers Quarry in eastern Tennessee received effluent from a coal-fired power plant (Southworth *et al.* 1994). The effluent contained high concentrations of Se that caused largemouth bass (*Micropterus salmoides*) to accumulate high Se concentrations. After Se concentrations decreased in Rogers Quarry, Hg concentrations in muscle of largemouth bass increased by eight times to concentrations that were comparable to background Hg concentrations in other systems.

Southworth *et al.* (2000) extended the data set in Southworth *et al.* (1994) to 1998 to assess whether Hg concentrations in muscle of largemouth bass had stabilized. There was no evidence that the rate of Hg bioaccumulation was slowing. Elevated Se concentrations in muscle of fish did not inhibit Hg concentrations in muscle from increasing.

Walleye (*Stizostedion vitreum*) fry were stocked in Lake Oahe, South Dakota, in 1983 to offset a decline in the walleye fishery (Mauk and Brown 2001). There were no significant correlations between concentrations of Se and Hg in muscle of adult walleye among sites in tributaries of Lake Oahe (Mauk and Brown 2001); however, there were significant negative correlations between Se concentrations in muscle and Hg concentrations in liver in males (r^2=0.08; p=0.03) and females (r^2=0.11; p=0.0099). Females also had significant negative correlations between Se concentrations in muscle and Hg concentrations in muscle (r^2=0.14; p=0.0036), Se concentrations in muscle and Hg concentrations in ovaries (r^2=0.09; p=0.0201), and Se concentrations in ovaries and Hg concentrations in ovaries (r^2=0.15; p=0.0186).

In 1996 and 1997, Chen *et al.* (2001) investigated the effect of Se on Hg concentrations in yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum*) in nine lakes with different Se concentrations in Sudbury, Ontario. Smelting of copper-nickel ores in Sudbury dispersed Se through atmospheric emissions and resulted in elevated Se concentrations in lakes closest to the smelter. There was a significant inverse relationship between Hg and Se concentrations in muscle of yellow perch (r^2=0.79, p<0.05) and walleye (r^2=0.97, p<0.01; (Chen *et al.* 2001). Concentrations of Hg in muscle of fish increased with increasing distance from the smelter, and correlated with decreasing Se concentrations in muscle of fish.

Belzile *et al.* (2006) continued the investigation initiated by Chen *et al.* (2001). There was a significant inverse linear regression between MeHg concentrations in muscle of yellow perch and total Se concentrations in water (r^2=0.59; p<0.05; Belize et al. 2006).

Concentrations of MeHg did not increase as quickly as total Hg with decreasing Se concentrations in water. With increasing distance from the smelter, Se concentrations in water decreased and the percent total Hg that occurred as MeHg decreased in organisms. Belzile *et al.* (2006) concluded that the percentage of total Hg that occurred as MeHg decreased with decreasing Se concentrations in water. Therefore, at higher Se concentrations in water the proportion of MeHg in muscle of fish was higher. This is important because it indicates that MeHg should be analyzed in Se and Hg studies because the proportion of MeHg in muscle may change with exposure to Se. Methyl Hg is a better comparison than total Hg for assimilation

studies because MeHg reflects biological assimilation, eliminates non-bioavailable forms, and is more sensitive in low Hg systems (Belzile *et al.* 2006).

Analyzing only total Hg may give the appearance that Se additions are decreasing Hg concentrations, but MeHg concentrations may not be significantly different. Therefore exposure to MeHg may not decrease significantly after Se addition even if it appears that total Hg concentrations in fish have decreased.

Synthesis

Froslie *et al.* (1985) reported no consistent correlations between Se and Hg in fish, but Speyer (1980), Leskinen *et al.* (1986), Southworth *et al.* (1994, 2000), Mauk and Brown (2001), and Chen *et al.* (2001) all found lower Hg concentrations in muscle of fish when Se concentrations in muscle were higher. This relationship was statistically significant in Leskinen *et al.* (1986), Mauk and Brown (2001), and Chen *et al.* (2001). Belzile *et al.* (2006) found no statistically significant relationship between Hg and Se concentrations in muscle tissue. Rather, they found that Hg concentrations in muscle of fish were significantly inversely correlated with Se concentrations in water. When Se concentrations were higher in water, Hg concentrations were lower in muscle. This suggests that Se was acting antagonistically on Hg bioaccumulation. Belzile *et al.* (2006) also found that the proportion of MeHg in muscle decreased with increasing Se concentrations in water, indicating that MeHg should be analyzed in Se studies rather than approximated by total Hg analysis.

Leskinen *et al.* (1986) and Mauk and Brown (2001) examined concentrations of Hg in muscle and liver. The muscle to liver ratio of Hg and Se was significantly higher in natural lakes compared to man-made lakes (Leskinen *et al.* 1986). Also, the proportion of MeHg in liver was lower when Se concentrations in liver were higher (Leskinen *et al.* 1986). Mauk and Brown (2001) found a significant negative correlation between Se and Hg concentrations in various tissues of fish. Belzile *et al.* (2006) found the proportion of MeHg in muscle of yellow perch increased with increasing Se concentrations in water despite a significant decrease in total Hg concentrations. Leskinen *et al.* (1986) and Belzile *et al.* (2006) show opposing effects to the proportion of MeHg, but the tissues analyzed were different; Leskinen *et al.* (1986) analyzed liver and Belzile *et al.* (2006) analyzed muscle. These studies show inconsistent correlations between Se and Hg concentrations in tissue of fish.

To investigate mechanisms of Se and Hg interactions in fish, Leskinen *et al.* (1986) and Mauk and Brown (2001) calculated the ratio of Hg concentrations in muscle and liver. Mauk

and Brown (2001) correlated Se and Hg concentrations in many fish tissues. Belzile *et al.* (2006) found a significant inverse correlation between MeHg concentrations in muscle of fish and Se concentrations in water.

Concentrations of Hg and Se in muscle of fish from rivers

Fish that inhabit rivers seem to exhibit either no correlation or positive correlations between Se and Hg concentrations in muscle of fish. The Madeira River, Brazil (Dorea *et al.* 1998), Piriá River, Brazil (Lima *et al.* 2005), and the Savannah River, Georgia (Burger *et al.* 2001) contained inconsistent significant positive correlations between Se and Hg in muscle of fish. These correlative studies did not indicate mechanisms, but they may indicate different relationships between Se and Hg among water systems.

Case Studies

Dorea *et al.* (1998) reported the concentrations of Se and Hg in seven fish species from the Madeira River in Brazil, which is contaminated with Hg from gold mining. Concentrations of Hg and Se in herbivorous fishes correlated positively and significantly (r^2=0.52; p=0.0088) but not for other feeding behaviours.

Burger *et al.* (2001) tested whether Hg and Se concentrations differed in muscle of fish that were caught upstream, at, and downstream of the Department of Energy, a nuclear weapon production site from the Savannah River in South Carolina and Georgia. Concentrations of Hg and Se in muscle of yellow perch (tau=0.33; p=0.003) and red-breasted sunfish (tau=0.33; p=0.006) showed a significant positive correlation, but no correlation was observed in the other species.

In 1999, 2000, and 2001, Lima *et al.* (2005) assessed Hg concentrations in fish species from the Piriá River and Lagoa Grande Reservoir in northern Brazil and evaluated correlations between Hg and Se concentrations. Concentrations of Hg and Se correlated positively and significantly in muscle of only two species: traíra (n=35, r^2=0.787, p=0.005) and aracu (n=38, r^2=0.485, p<0.005), which were the only two species with sample sizes larger than seven.

Synthesis

No mechanisms of Hg and Se interactions were explained in these studies. In rivers, usually no relationships existed between Hg and Se concentrations in muscle of fish. In rivers in the southeast United States and Brazil, positive correlations between Hg and Se concentrations in muscle of fish were observed in a few species. Concentrations of Hg and Se in yellow perch in the Savannah River differed from lakes in temperate to boreal climates. *Leporinus sp.* was present in the Savannah and Piriá Rivers and both locations exhibited positive correlations between Hg and Se concentrations in muscle.

The ratio of Hg to Se increased with trophic level. In the Piriá River, the ratio of Hg to Se increased from 0.14 in non-piscivorous to 0.65 in piscivorous fish. In the Madeira River, the ratio of Hg to Se increased from 0.9 in herbivorous to 1.9 in omnivorous to 4.0 in piscivorous fish (Dorea *et al.* 1998). Although the range of Hg to Se in fish muscle is quite different between the Piriá and Madeira rivers, both exhibit increasing ratios of Hg to Se from non-piscivorous to piscivorous fish.

Effects of Se on Hg toxicity in fish

Lowering Hg exposure would translate to lower Hg toxicity to fish, their eggs, and consumers of fish, but none of the studies reviewed thus far indicated whether Se lowers Hg toxicity to fish. The studies in this section give inadequate evidence for Se lowering Hg toxicity in the minnows and fish eggs.

There is evidence for synergistic toxicity of Hg and Se. Synergistic toxicity is when the toxicity of two elements together is greater than the toxicity of individual elements. Synergistic toxicity may also be referred to as additive effects. In other words, the toxicity of Hg may be enhanced by Se.

There is also evidence of Se antagonizing the effects of Hg by increasing the survival time of fish exposed to Hg or increasing hatchability. Antagonistic toxicity is when the effects of Hg and Se together are less than effects due to Hg alone. In the case study below Se also increased Hg concentrations in fish tissue.

Case Studies

Huckabee and Griffith (1974) studied the effects of Hg and Se to carp eggs using a hatching test. The duration of the experiment was the time to hatch and the endpoint was percent hatch. Hg toxicity impacts due to adding Se to carp eggs were synergistic. At 4 μg/L

mercuric chloride, no carp eggs hatched and therefore mercuric chloride significantly affected hatchability at 4 µg/L ($p<0.05$). Hatchability was not affected at 5 µg/L of selenite and no mercuric chloride. Percent hatch was lower when mercuric chloride and Se were combined.

Klaverkamp *et al.* (1983b) evaluated toxic interactions of Hg and Se on fertilized rainbow trout (*Salmo gairdneri*) and lake trout (*Salvelinus namaycush*) eggs. Sodium selenite had no effect on Hg toxicity to rainbow and lake trout eggs, except at extremely high concentrations. Concentrations of sodium selenite that antagonized Hg toxicity were so unrealistically high that we consider the results of this study to indicate that Se has no effect on Hg toxicity to rainbow and lake trout eggs at environmentally realistic concentrations.

Cuvin and Furness (1988) examined whether Se protects against mercuric chloride toxicity in the minnow (*Phoxinus phoxinus*). Selenite significantly increased the survival rate of minnows exposed to mercuric chloride. Exposure to Se in combination with Hg showed higher survival rates of minnows than those exposed to Hg alone. Twice as much Se as Hg was most effective in increasing survival of Hg exposed minnows. Although Se increased their survival when exposed to Hg, minnows in Se treatments actually acquired significantly greater concentrations of Hg, but the reason for this was not reported. Selenium increased Hg uptake from water into minnows, but it also increased survival.

Siegel *et al.* (1991) collected tilapia (*Mossambicus oreochromis*) from a reservoir in Hawaii to test the ability of Se to prolong life when the fish were exposed to Hg. Tilapia exposed to sodium selenite and mercuric chloride had significantly higher survival than those in the control, which contained only mercuric chloride ($p<0.02$). Survival of tilapia exposed to sodium selenate and mercuric chloride was not different from the control.

Synthesis

Synergistic effects between Hg and Se were found in carp eggs by Huckabee and Griffith (1974), but not in rainbow and lake trout eggs by Klaverkamp *et al.* (1983b). Klaverkamp *et al.* (1983b) stated that their results contradict Huckabee and Griffith (1974). The major differences were in experimental procedures, temperature, duration, life stage, salinity, and species. Huckabee and Griffith (1974) ran their experiment for a longer duration in saline solution and at warmer temperatures than did Klaverkamp *et al.* (1983b). We consider that neither study indicates antagonistic effects of Se on Hg toxicity to eggs of fish because concentrations used were unrealistically high.

Although Cuvin and Furness (1988) and Siegel *et al.* (1991) showed that survival of Hg exposed fish increased with exposure to Se, their data do not indicate that symptoms of toxicity were alleviated. Selenium increased the survival rate in the minnow (Cuvin and Furness 1988) and tilapia (Siegel *et al.* 1991) exposed to waterborne mercuric chloride; however, Se also increased Hg uptake in minnows (Cuvin and Furness 1988). Toxicity tests conducted with the minnow and tilapia were done at unrealistically high concentrations of both Hg and Se. These toxicity tests also used only waterborne exposure to Hg and Se, while the main route of exposure for both Hg and Se is via food. The value of these toxicity tests is limited because of the unrealistic concentrations, unrealistic routes of exposure, and short durations. In their experiment, Cuvin and Furness (1988) exposed fish to 300 µg/L of Hg and 100 to 600 µg/L of Se. The fact that Se did increase survival of Hg exposed organisms indicates further testing could be done for longer durations at lower concentrations of Hg and Se in food and water to examine toxicity endpoints in tissues, such as gonads.

Se increases the loss of Hg from fish

Selenium may cause MeHg to break down into inorganic Hg and a methyl group, and result in relocation of Hg within the fish or increase the loss of Hg through feces or urine (Bjerregaard *et al.* 1999). There are no definitive case studies that explain how this mechanism works but there are general indications that Se can cause some remobilization of Hg. The first study that will be reviewed assessed the effects of dietary Se on Hg concentrations in muscle and organs of rainbow trout (Bjerregaard *et al.* 1999). The second study addressed uptake and loss of Hg through the gills of rainbow trout (Pedersen *et al.* 1998).

Case Studies

Bjerregaard *et al.* (1999) investigated the effect of dietary Se on the retention of organic and inorganic Hg in juvenile rainbow trout (*Oncorhynchus mykiss*). Rainbow trout that were fed 10 µg/g of Se for six weeks eliminated significantly more MeHg from muscle ($p<0.001$), liver ($p<0.001$), kidney ($p<0.001$), and erthrocytes ($p<0.05$), but not in bile or plasma compared to those in control treatments. Dietary Se supplements significantly lowered inorganic Hg concentrations in muscle ($p<0.05$), kidney ($p<0.01$), and erthrocytes ($p<0.05$), but not in liver, bile, or plasma compared to those in control treatments. Bjerregaard *et al.* (1999) hypothesized that dietary selenite may bind MeHg and lead to elimination via feces instead of distribution into tissue.

Another route of exposure, and thus another site for potential Hg and Se interactions, is through gills. Absorption of Hg through gills was found to be affected by gill permeability of rainbow trout (*Oncorhynchus mykiss*), which varied with treatment (Pedersen *et al.* 1998). Selenium uptake by gills of rainbow trout occurred slowly. Uptake of MeHg into gills from water increased three times in selenite treatments of 1889 μg/L (7.5 μM; $p<0.001$). Selenite increased the release of MeHg from gills ($p<0.001$). Because food is the main source of Hg and Se to fish, we assume the exchange of Hg through gills will be less significant even at elevated concentrations of Se.

Synthesis

Reviewed studies provided some evidence for Hg loss from tissues in the presence of Se. In the presence of Se, Bjerregaard *et al.* (1999) found Hg was released from liver and kidney while Pedersen *et al.* (1998) found uptake and loss of Hg through dismembered gills. If Se concentrations in blood were elevated, then perhaps Hg taken up through diet could be lost through gills. Waterborne and dietary Se may have affected the amount and chemical form of Se in tissues of fish and therefore these two routes of exposure may have affected Hg differently. Timing of respective exposure, dosage, form, and route of exposure may all have affected Hg and Se interactions. Further research would be required to evaluate these ideas.

Se may lower the rate of Hg methylation in aquatic environments

Concentrations of MeHg in fish depend on the amount of MeHg available for uptake, among other factors. If the amount of Hg that becomes methylated is lowered, there would be less MeHg available for bioaccumulation. The rate of Hg methylation depends partly on the ionic Hg concentrations in sediment. Selenium may lower Hg methylation because Se anions can bind with Hg cations and form inert mercuric selenide. In this case, the rate of Hg methylation would depend on the rate of selenide reduction (Chen *et al.* 1997). Mercuric selenide is not likely taken up by microorganisms and therefore probably cannot be microbially methylated.

Case Studies

Jackson (1991) investigated effects of Se on MeHg concentrations in lake sediment. This study deduced that MeHg was not produced at high Se concentrations because of the

formation of insoluble mercuric selenide, not by inhibition of biological processes. He concluded that to suppress MeHg by adding Se, toxic concentrations of Se would be required.

Jin *et al.* (1999) studied the effect of Se on Hg methylation in anaerobic surface sediment from Donghu Lake, China. There was a significant difference among three groups of treatments: high MeHg concentrations were measured in aerobic treatments without Se and anaerobic treatments with 0.25 μg/g of Se; medium concentrations of MeHg were measured in anaerobic treatments without Se; and low concentrations of MeHg were measured in anaerobic treatments with 2.5 and 5 μg/g of Se. Therefore, in anaerobic sediment, low concentrations of Se (0.25 μg/g) stimulated Hg methylation, while the rate of Hg methylation was lower at 2.5 and 5 μg/g of sodium selenite.

The experimental design of Jin *et al.* (1999) could have been improved by lowering the amount of mercuric chloride and the incubation temperature to realistic levels. Even though the concentrations of mercuric chloride and the temperature of incubation were high, the results of Jin *et al.* (1999) were similar to the other two studies (Chen *et al.* 1997; Jackson 1991).

Chen *et al.* (1997) estimated the effect of sodium selenate and selenite and sulphate on Hg methylation rates and on microbial activity in sediment from 0 to 5 cm depth from the Carson River-Lahontan Reservoir, Nevada. Significant inhibition of Hg methylation occurred at 34 μg/L (>270 nM) of selenate. Selenate at 189 μg/L lowered Hg methylation by 94% relative to controls. Selenite concentrations between 0.08 to 127 μg/L (0.65 to 1000 nM) consistently lowered the rate of Hg methylation by 40% at all concentrations of Se. No significant differences in microbial activity resulted between treatments, although the microbial activity was lower when Se was added. Therefore Chen *et al.* (1997) suggested there was general microbial toxicity from selenate. Sulphate increased the suppression of Hg methylation by selenate (an additive effect). Thus they suggested the mechanism by which selenate lowered Hg methylation was similar to that which caused sulphur to lower Hg methylation. This process was the formation of insoluble complexes with Hg. Therefore the lower rate of Hg methylation may have depended on the rate of Se reduction to selenide (Chen *et al.* 1997).

Synthesis

Jackson (1991) found MeHg concentrations in sediment were lowered at Se concentrations less than 790 μg/L, and MeHg was not detected when Se concentrations were more than 3,948 μg/L. Jin *et al.* (1999) found that methylation of Hg was stimulated at 0.25 μg/g of sodium selenite and lowered at sodium selenite concentrations of 2.5 and 5 μg/g. Chen

et al. (1997) found significantly lower Hg methylation at 34 µg/g (>270 nM) of selenate. Methyl Hg was lowered by 94% at 189 µg/L of selenate and by 40% at 127 µg/L of selenate (Chen *et al.* 1997). Selenite at 0.08 to 127 µg/L (0.65 to 1000 nM) consistently lowered the rate of Hg methylation (Chen *et al.* 1997). Jackson (1991) and Jin *et al.* (1999) both found that low concentrations of Se could stimulate Hg methylation. Chen *et al.* (1997) found that Se consistently lowered Hg methylation but not always significantly. Regardless of the inconsistencies, the concentrations of selenite or selenate required to significantly lower Hg methylation could become toxic to fish.

Another conclusion from these studies pertains to the mechanism by which Se is thought to lower Hg methylation. Jackson (1991) suggested that the formation of insoluble mercuric selenide lowers the ability of microorganisms to convert Hg to MeHg. Presumably, microorganisms cannot take up or metabolize mercuric selenide. Jackson (1991) deduced that microorganisms were not affected by selenite because he observed carbon dioxide production was unaffected by increased Se concentrations, which he interpreted to indicate metabolism of microorganisms. Jin *et al.* (1999) suggested that sulphide binds Hg in anoxic sediment, but did not cause the formation of mercuric selenide. Chen *et al.* (1997) tested the effect of sulphide and selenate on Hg methylation, which lowered Hg methylation more than selenate alone. Chen *et al.* (1997) suggested the mechanism by which selenate lowered Hg methylation is the same by which sulphide lowers methylation, by forming a stable complex with Hg. In conclusion, Se is thought to lower Hg methylation by making Hg unavailable to methylating microorganisms.

CAN SE ADDITIONS SIGNIFICANTLY LOWER HG CONCENTRATIONS IN FISH?

When assessing the usefulness of Se to lower Hg concentrations in fish, we need to define how much lower the Hg concentrations need to be. One way to define this is by lowering concentrations of Hg in muscle of fish to the advisable safe consumption guideline. The Canadian guideline for commercial sale of fish is 0.5 µg/g (wet weight) of Hg. As just one example, fish in hydroelectric reservoirs often acquire up to 1.5 µg/g of Hg in muscle. Lowering Hg concentrations in muscle of fish from 1.5 to 0.5 µg/g is a 66% reduction. Assuming that effects are smilar regardless of Hg concentrations, we assessed whether past studies indicated whether Se could lower Hg concentrations by 66%, or to reach <0.5 µg/g. We did this by running linear regressions to analyze data from whole-lake studies undertaken in contaminated lakes in Sweden where pre-treatment Hg concentrations in northern pike were a mean of 1.2 and a maximum of 2.2 µg/g (Paulsson and Lundbergh 1991). According to the regression, more

than 3 μg/L of sodium selenite would be required to lower the Hg concentrations in northern pike by 66% (Figure 1). It is not known if Hg concentrations in fish will decrease by the same percentage independent of the initial Hg concentration in fish.

It is unacceptable to add 3 μg/L of Se to lakes because this water concentration has been shown to cause chronic toxicity to fish (Lemly 2002a). Therefore substantially lowering Hg concentrations in muscle of fish by Se addition would very likely elevate the risk of Se toxicity to fish. Because 1 μg/L of Se gave 20% lower Hg concentrations in muscle of yellow perch, future research should consider how a 20% reduction seen at 1 μg/L of Se would translate to higher trophic levels. Turner and Rudd (1983) predict that piscivorous fishes will have greater reduction in Hg bioaccumulation at low Se concentrations than detritivorous and omnivorous fishes. Because Se efficiently bioaccumulates, Rudd and Turner (1983) suggested that maintaining elevated aqueous Se concentrations would not be necessary. However, in Sweden the percent reduction of Hg concentrations in muscle of northern pike was less than that in fish at lower trophic levels, though these concentrations were not at steady state. In addition, bioaccumulation of Se in muscle of fish at higher trophic levels was not as great as those at lower trophic levels (Paulsson and Lundbergh 1991).

TOXICITY OF SE

As an essential element, Se is acquired through diet, and as a component of amino acids, Se supports protein synthesis and antioxidation. Although fish can take up some Se through their gills (Pedersen *et al.* 1998), diet is the main route of Se into fish, and the efficacy of bioaccumulation depends largely on the structure of the food web (Baines *et al.* 2002). In addition, the degree to which Se bioaccumulates and biomagnifies is specific to the organ tissue. Selenomethionine is the most readily bioaccumulated form of Se, followed by selenite and then selenate. Fish require 0.1 to 0.5 μg/g (d.w.) in their diet (Hodson and Hilton 1983), which is used to create glutathione peroxidase, an antioxidant enzyme that protects cell membranes (Lemly 1998b) and T_4ORD deiondinase that converts the inactive form of thyroid hormone to the active form (Köhrle 1996). Selenium deficiency in fish is recognized by reduced growth, anemia, muscular dystrophy, and death (Lemly 1998b).

The toxic threshold for Se can be surpassed at concentrations slightly above those which are essential. In the diet of fish, concentrations of Se greater than 3 μg/g (d.w.) may become poisonous. In eggs, 10 μg/g (d.w.) of Se may cause malformations. In addition, the USEPA (2004) set the aquatic life criterion for tissues of fish at 7.9 μg/g (d.w.) of Se and if it exceeds

5.8 µg/g (d.w.) of Se during summer or fall, fish should be monitored throughout the winter. In British Columbia, Canada, the guidelines for Se are mean concentrations of 2 µg/L in water, 2 µg/g (d.w.) in sediment, and 1 µg/g (w.w.; or about 5 µg/g d.w.) in tissue of fish (Nagpal 2001).

One of the endpoints of Se toxicity in fish is the reproductive system. Chronic Se exposure can compromise reproduction without affecting survival of adult fish. It transfers from the liver to the gonads of the adult fish via vitellogenin during oogenesis and excess Se is deposited in the yolk of the eggs as selenoamino acids, selenomethionine and selenocystine (Lemly 2002a). Vitellogenin is a lipophosphoprotein-calcium complex (Lemly 1998a), which is synthesized in the liver. The liver releases vitellogenin into the blood during oogenesis. In the gonads, oocytes sequester vitellogenin from blood and store it in the yolk sack. Yolk incorporation into the cytoplasm is called vitellogenesis. Larvae use the yolk for an energy and protein source to build hard and soft tissues.

At threshold concentrations for congenital malformations, survival of adult fish and hatchability of eggs may not be affected (Lemly 1998b). In developing larvae, however, too much Se results in dysfunctional proteins or enzymes. Proteins are normally built in a helix structure because of disulfide bonds between amino acids. Excess Se replaces sulfur as triselenium or selenotrisulfide (Lemly 2002a). Selenium has the same valence states as sulfur and forms similar compounds like selenite, selenate, and hydrogen selenide. This substitution of Se for sulphur changes the helix structure and disrupts either tissue synthesis or cellular metabolism (Lemly 2002a). Selenium affects the biogeochemical, cellular, and organ systems, and the effects can range from congenital malformations to death. The main environmental effect of selenosis is failure to recruit because of larvae mortality when deformities affect feeding, escape of predation, or respiration (Hamilton 2004).

Not all symptoms of selenosis can be explained by Se substitution into sulphur bonds during protein synthesis. Some studies have shown lesions can result from excess Se. The presence of lesions is indicative of oxidative damage. Peak activity for generating superoxide from selenomethionine occurs after early liver development (Palace *et al.* 2004b). In addition, haemorrhaging in rainbow trout larvae occurs when there is fluid retention in the yolk sac and heart tissue. Fluid retention can also be caused by oxidative stress.

The most noticeable signs of congenital Se poisoning are deformations of the skeleton, fins, head, and mouth (Lemly 1993; Lemly 1997). Acute toxic responses are edema (swollen and descended abdominal and visceral cavities), exophthalmia (bulging eyes from fluid retention in the eye sockets), and cataracts (white coating over eyes). Spine curvature may occur in three

forms: lordosis is the concave curvature of the lumbar spine; kyphosis is convex curvature of the thoracic spine; and scoliosis is lateral curvature of the spine. Fins, gills, and eyes may be missing or deformed, and the head and mouth may be malformed. Cell permeability increases because selenoproteins in the cell membrane allow excess fluid to enter organs. However, aqueous Se did not alter the uptake of Hg in the gills of rainbow trout (Pedersen *et al.* 1998).

Less obvious symptoms of selenosis can be seen in the blood and organs. The gills may have dilated blood sinusoids, impaired blood flow, ineffective gas exchange, metabolic stress response, swollen lamellae (telangiectasia), erythrocytes, and hemorrhaging of tissue. The blood may have reduced hematocrit values, elevated lymphocytes, lower immune response, fewer hemoblasts, reduced erythropoiesis, and delayed replacement of red cells. The liver may have lymphocyte infiltration, vacuolization of parenchymal hepatocytes, swelling, deformed cell nuclei, and perisinusoidal lipid droplets. The kidney may have focal intracapillary proliferative glomerulonephritis, periglomerular fibrosis (which leads to tissue hardening), and tubular epithelium. The heart may have inflamed cells in pericardial space (pericarditis) and in ventricular myocardial tissue (myocarditis). The ovaries may be swollen, necrotic, or ruptured (Lemly 1997). Signs of congenital malformations are usually diagnosable by eye, but a microscope may be required for small larvae (Lemly 1998a).

In threatened species of fish, non-lethal samples of muscle tissue have been used to estimate the potential for selenosis in fish embryos and larvae. There is a strong correlation between Se concentrations in the muscle and eggs of razorback suckers (Hamilton and Waddell 1994), rainbow trout, and brook trout (Holm *et al.* 2003). The correlation between Se concentrations in muscle and eggs is species specific (Palace *et al.* 2004a). The limitations of using muscle to estimate the potential for selenosis in fish offspring are small tissue mass, effects from other elements, and short biological half-lives in tissue, as well as differences in the relationships between the two tissue types at different times of the reproductive cycle (Palace *et al.* 2004a).

Synthesis from case studies

The risk of Se toxicity is a critical consideration regarding Se additions to lower Hg in fish. Although acute toxicity was thoroughly tested by Rudd *et al.* (1980), they did not address chronic toxicity. Rudd *et al.* (1980) observed that 100 μg/L of sodium selenite lowered Hg bioaccumulation by 50%; however, these concentrations are far greater than those that have caused Se toxicity. Concentrations of 10 μg/g of Se in the diet of fish for eight weeks caused Se

concentrations in liver to increase from 1 to 26 μg/g (Bjerregaard *et al.* 1999). This dietary Se exposure is higher than expected in natural systems and exceeds the recommended threshold of 3 μg/g in prey of fish to prevent toxicity (Lemly 2002a).

In a pond receiving Se-rich fly ash Se in muscle of fish ranged from 3 to 35 μg/g (w.w.; 15 to 18 μg/g d.w.). Gross abnormalities of incomplete and deformed boney tissue in head, fins, and gill covers were observed in about 30% of the fish collected (Southworth *et al.* 2000). Two years after cessation of effluent, Se concentrations in muscle of fish ranged from 1 to 1.5 μg/g (w.w.; 5 to 7.5 μg/g d.w.) and deformities in offspring were not evident. Chen *et al.* (2001) also observed many teratogenic defects in fish from lakes in Sudbury. In addition, shallow lakes with poor circulation and thick ice had Se concentrations that reached 35 μg/L during the second year of additions in the Swedish studies. No yellow perch were caught in four small lakes (Paulsson and Lundbergh 1991), perhaps due to winter stress syndrome or failure to recruit. Paulsson and Lundbergh (1991) suggested keeping water concentrations of Se under 2.0 μg/L and adding Se during periods of high water circulation.

In Paulsson and Lundbergh (1991), the increase of Se concentrations in muscle of fish correlated with an increase of Se concentrations in liver and gonads of fish. Mauk and Brown (2001) found a significant positive correlation between Se concentrations in gonads and Se concentrations in muscle of walleye at spawning ($p=0.0001$; $r=0.49$). Holm *et al.* (2005) also found a significant positive relationship between concentraitons of Se in gonads and muscle in rainbow and brook trout. These relationships are species specific and would vary with time of sampling relative to spawning. Using these relationships to monitor Se concentrations in fish using non-lethal methods (Baker *et al.* 2004) is possible, but should be treated with caution. Non-lethal monitoring of Se concentrations in fish is also discussed in Lemly (2002a).

CONCLUSION

The literature we reviewed indicates lower Hg concentrations in muscle of fish are often associated with elevated Se concentrations. However, it is unresolved how Se lowers MeHg concentrations in freshwater fish. The most well-supported mechanism for Se lowering Hg is by modifying bioaccumulation of Hg in muscle of fish (Paulsson and Lundbergh 1989; Paulsson and Lundbergh 1991; Rudd *et al.* 1980; Turner and Rudd 1983; Turner and Swick 1983). In addition, Se may increase Hg removal from fish (Bjerregaard et al. 1999). At high concentrations, Se lowers microbial methylation of Hg, but these concentrations of Se would result in Se toxicity in fish. At low concentrations of Se, methylation of Hg was enhanced

(Jackson 1991; Jin *et al.* 1999). The relative importance of the mechanisms by which Se lowered concentrations of total Hg and MeHg in fish is unknown.

Many of the case studies of Se and Hg interactions showed significant inverse correlations between Hg and Se in muscle of fish. In river systems, there usually were no relationships between Hg and Se concentrations in muscle of fish, although a few species inhabiting rivers had significant positive correlations between Hg and Se concentrations in muscle. Most research on Hg and Se interactions in fish in lakes report inverse correlations. Correlative studies point to differing interactions between Hg and Se in lakes and rivers, without determining the mechanisms.

Knowledge of the physiological distribution of Se and Hg in organisms and the biochemical mechanisms of Hg and Se interactions is essential to determining exposure to Hg. It may be worthwhile for humans to lower Hg in muscle of fish by adding Se but this may not lower the exposure of Hg to other fish and wildlife. If Se only redistributes Hg from muscle into the liver or kidney, wildlife may be exposed to Hg if they eat these organs. Moreover, wild animals may become exposed to both Hg and Se. In addition, if the proportion of total Hg that occurs as MeHg in muscle of fish does not change, human Hg exposure will not be lower.

In future studies of Hg and Se interactions, environmentally relevant chemical forms and concentrations of Hg and Se are imperative. The most important endpoint for Se toxicity is reproduction (teratogenesis) which occurs at much lower concentrations than acute toxicity. In addition, the main route of exposure to Se and Hg is through diet (Turner and Swick 1983). Therefore dietary and waterborne exposure should be used when testing the ability of Se to lower Hg concentrations in fish. Because Se may affect inorganic Hg in fish more than MeHg (Belzile *et al.* 2006), MeHg should be analyzed in fish tissues, not just total Hg as an approximation for MeHg. Using total Hg as an approximation for MeHg only is valid when most of the Hg in fish is present as MeHg (Bloom 1992). Whenever possible, liver and muscle should be analyzed because liver seems to contain the highest Se concentrations and muscle contains the highest Hg concentrations. Also, tissue samples should be dried to decrease variation due to water retention.

Future research required to fill in the gaps in our knowledge are: 1) what is the lowest concentrations of Se additions that will lower MeHg concentrations in fish without causing toxicity? 2) how does adding Se affect food webs of different lengths? 3) does Se bioaccumulation to higher trophic levels require lower Se concentrations, or fewer Se additions, to lower MeHg concentrations to meet consumption guidelines? 4) for how long are Se additions

required to maintain lower Hg concentrations in fish? and 5) what is the relative importance of the mechanisms by which Se lowers MeHg in fish?

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Table 1. Summary of Se and Hg interaction studies.

Mechanism	Details	Findings	Criticisms	References
Lower bioaccumulation				
	Pearl dace Clay Lake, Ontario Mesocosms	100 µg/L selenite lowered ^{203}Hg uptake Se detected in food web after 2 weeks	Toxic Se concentrations Not replicated Se uptake rate 0.1 µg/g*day	Rudd *et al.* 1980
	Pearl dace, yellow perch, white sucker Clay Lake, Ontario Mesocosms	1 µg/L selenite lowered ^{203}Hg uptake by 20% in fish ^{203}Hg uptake not lower in lower food web	10 and 100 ug/L Se treatments toxic not replicated Se uptake rapid	Turner and Rudd 1983
	Northern pike Clay Lake, Ontario Mesocosms	10 µg/L selenite and Se supplemented food lowered Hg uptake by 30 to 60%; food only lowered Hg uptake by 50%	Toxic Se concentrations Short duration	Turner and Swick 1983
	Northern pike Ontario Microcosms	LC_{50} 11,000 µg/L selenite 1 µg/L Se lowered Hg in carcass and muscle plus skin 100 µg/L of Se increased Hg in carcass	10 and 100 ug/L Se toxic Short Exposure through water only	Klaverkamp *et al.* 1983a
	Roach, yellow perch, northern pike Lake Oltarjain, Sweden Whole lake	5 µg/L selenite lowered yellow perch Hg 84% and roach and northern pike Hg 90%	Not replicated, but realistic Se bioaccumulated to 12 µg/g in roach, 15 µg/g in yellow perch, and 4 µg/g in northern pike	Paulsson and Lundbergh 1989
	Yellow perch and northern pike	1-2 µg/L selenite lowered Hg 60% and 3-5 µg/L selenite lowered Hg by 85%	Se bioaccumulated to 3.5 to 7 µg/g in yellow perch	Paulsson and Lundbergh 1991

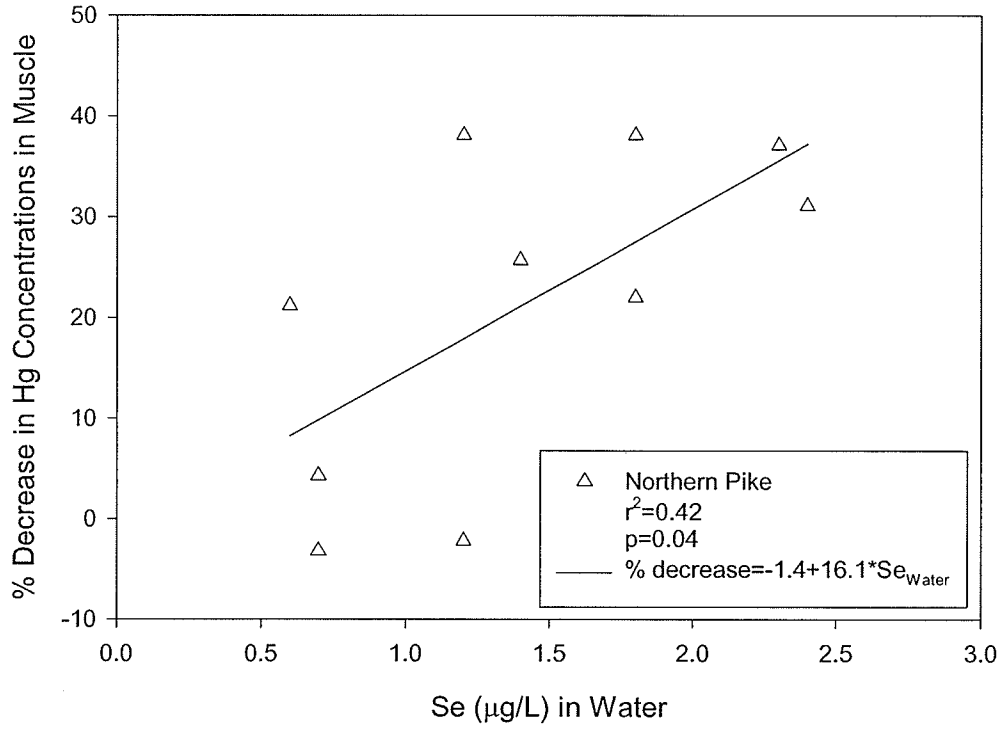
	11 lakes, Sweden	in yellow perch		
	whole lakes	northern pike Hg lowered 22-28%		
Decrease Hg concentrations				
	Northern pike Lakes Dufault and Duparquet, Quebec	Lake Dufault had high Se and low Hg in muscle Lake Duparquet had low Se and High Hg in muscle	Correlation, not causation	Speyer 1980
	Cisco, brown trout, smelt, burbot, yellow perch, northern pike 6 lakes in Norway	Positive correlation between Hg and Se in yellow perch muscle from 2 lakes	Correlation, not causation Small sample sizes	Froslie *et al.* 1985
	Northern pike Reservoirs and natural lakes in Finland	Negative correlation between Hg and Se in muscle when all lakes combined Hg:Se 4.72 reservoirs and 0.98 natural lakes	Correlation, not causation	Leskinen *et al.*1986
	Largemouth bass Rogers Quarry, Tennessee 1990-1993	Se water decreased from 25 to <2 µg/L Hg increased 0.02 to 0.17 µg/g in three years (8-times)	Correlation, not causation	Southworth *et al.* 1994
	Largemouth bass Rogers Quarry, Tennessee 1993-1998	Muscle Se remained two times greater than background Muscle Hg increases from 0.02 to 0.61 µg/g	Correlation, not causation Hg uptake not slowed by Se in muscle	Southworth *et al.* 2000
	Walleye Lake Oahe, South Dakota	Negative correlation between liver Hg and muscle Se in males and females Negative correlation between muscle Hg and Se in females	Correlation, not causation	Mauk and Brown 2001
	Yellow perch and walleye	Negative correlation between Hg and Se in muscle of yellow	Correlation, not causation	Chen *et al.* 2001

	9 lakes, Sudbury, Ontario	perch and walleye		
	Yellow perch and walleye 9 lakes, Sudbury, Ontario	Negative correlation between muscle MeHg and water Se MeHg decreased with decreasing Se concentrations in water	Correlation, not causation	Belzile *et al.* 2006
Increase Hg concentrations				
	Madeira River, Brazil	Hg and Se in herbervous fish correlate positively Hg:Se increase with feeding type	Correlation, not causation	Dorea *et al.* 1998
	Savannah River, South Carolina and Georgia	Positive correlation between Hg and Se in muscle of yellow perch and red-breasted sunfish	Correlation, not causation	Burger *et al.* 2004
	Piria River, Brazil	Positive correlation between Hg and Se in muscle of tracira and aracu Hg:Se increased with feeding type	Correlation, not causation	Lima *et al.* 2003
Hg toxicity				
	Carp eggs Hatching test	5 µg/L selenite combined with HgCl decreased % hatch	Unnaturally high Se concentrations and unnatural exposure route	Huckabee and Griffith 1974
	Rainbow and lake trout eggs Hatching test	10,000 µg/L of Se had no effect 100,000 and 200,000 µg/L decreased Hg in lake trout only	Unnaturally high Se concentrations and unnatural exposure route	Klaverkamp *et al.* 1983b
	Minnows Survival test	Se increased survival of minnows exposed to Hg 2-times more Se than Hg most effective Minnows acquire	Se concentrations toxic Exposure route unrealistic Not replicated	Cuvin and Furness 1988

		more Hg in presence of Se		
	Tilapia Hawaii	Selenite significantly increased survival of Hg exposed tilapia	Se concentrations unnaturally high and unrealistic exposure route	Siegal *et al.* 1991
Increased loss				
	Rainbow trout Denmark	10 µg/g Se in diet lowered MeHg from liver, bile, muscle, erthrocytes, and kidney	Supplements in diet; not natural food	Bjerregaard *et al.* 1999
	Rainbow trout detached gills 60 minute load 60 minute clear	19 µg/L Se did not affect MeHg exchange via gills 1190 µg/L Se increased MeHg uptake and release via gills	Unnaturally high Se concentrations Dismembered organ Exposure route not most important	Pedersen *et al.* 1998
Lower methylation				
	Sediment	0-790 µg/L Se enhanced and depressed Hg methylation >790 µg/L Se depressed Hg methylation	Toxic Se concentrations	Jackson 1991
	Donhu Lake, China	Aerobic no Se high MeHg Anaerobic 0.25 µg/L Se high MeHg Anaerobic no Se medium MeHg Anaerorbic 2.5-5 µg/L Se lower MeHg	Amount of HgCl unrealistically high Temperature too high	Jin *et al.* 1999
	Carson River-Lahontan Reservoir, Nevada 0-5 cm	Selenite 0.08-127 µg/L lowered Hg methylatioin 40% Sulphate and selenate additive suppression of Hg methylation		Chen *et al.* 1997

FIGURES

Figure 1. Regression analysis of selenium concentrations in water and percent reduction in mercury in muscle of northern pike. Data from Paulsson and Lundbergh (1991). This system was not at steady state.



ARE CONCENTRATIONS OF SELENIUM LOWER IN RESERVOIRS AND DOES SELENIUM AFFECT MERCURY CYCLING? RESULTS FROM A RECONNAISSANCE SURVEY

ABSTRACT

We hypothesized that selenium (Se) may be lower in hydroelectric reservoirs than in natural lakes because decomposition of flooded organic matter creates oxygen-poor conditions that promote Se reduction to volatile dimethyl selenide. Because Se is known to lower mercury (Hg) bioaccumulation, lowered Se concentrations in new reservoirs may contribute to elevated Hg concentrations in fish. Our main objective was to test if Se concentrations were lower in hydroelectric reservoirs as compared to reference systems. We also assessed whether Hg and Se concentrations were correlated in water, soil, and muscle of fish, and methyl mercury (MeHg) in water, soil, zooplankton, emerging insects, and Chironomid larvae. We sampled three hydroelectric reservoirs and two reference lakes in northern Québec, and four experimental reservoirs and four reference lakes at the Experimental Lakes Area (ELA) in northwestern Ontario. Muscle of finescale dace had significantly lower Se concentrations in the ELARP (Experimental Lakes Area Reservoir Project) reservoir compared to Reference Lakes 115 ($p<0.0001$) and 632 ($p=0.0003$). Also, muscle of finescale dace had significantly lower Se concentrations in the FLUDEX (Flooded Upland Dynamics Experiment) reservoirs 1 and 2 compared to those in Reference Lake 115 ($p=0.0015$ and 0.0081, respectively). Muscle of finescale dace in FLUDEX reservoirs 2 and 3 had significantly higher Se concentrations compared to those in Reference Lake 632 ($p=0.023$ and $p<0.0001$, respectively). In Caniapisceau Reservoir, Se concentrations were significantly lower in muscle of longnose sucker ($p=0.0006$), lake whitefish ($p=0.0003$), and northern pike ($p<0.0001$) compared to those in reference lakes, while they were significantly higher in lake trout ($p=0.0081$). In Laforge-1 Reservoir, Se concentrations were significantly lower in longnose sucker ($p<0.0001$), lake whitefish ($p=0.0033$), and northern pike ($p<0.0001$) than those in the reference lakes. In Robertson Reservoir, Se concentrations in muscle of fish were significantly lower than those in the reference lake in rainbow smelt ($p=0.0036$) and brook trout ($p=0.0012$), those in arctic char were significantly higher ($p=0.0467$), and there was no significant difference for those in landlocked salmon. Throughout warmer months, Se concentrations in emerging insects, Chironomid larvae, and zooplankton were generally lower in reservoirs compared to those in reference lakes. Concentrations of Se were consistently lower in water from the ELARP

reservoir that was immediately downstream from its reference lake. Reference lakes in Québec did not flow directly into the reservoirs and the Se concentrations in water in these reservoirs were not consistently lower than those in reference lakes. Concentrations of Se in flooded and unflooded soil were not significantly different (p-values ranged from 0.34 to 0.98) except in FLUDEX reservoir 3 ($p=0.05$). There were no correlations between Hg and Se concentrations in zooplankton and emerging insects at the ELA or in zooplankton and Chironomid larvae in Québec. In small fish at the ELA, there seemed to be lower Hg concentrations in muscle of fish when Se concentrations in muscle of fish were higher. In all areas in Québec, there was no relationship between Hg and Se concentrations in muscle of fish. In summary, our results did not consistently support our hypothesis that Se is depressed in reservoirs.

INTRODUCTION

It has been hypothesized that concentrations of selenium (Se) may be lower in new reservoirs because of Se reduction and volatilization (H. Hultberg, pers. comm., IVL, Sweden). Flooding of terrestrial plants and soil causes microbial decomposition that can lower oxygen concentrations in the bottom waters of hydroelectric reservoirs. In anoxic conditions, oxidized forms of Se can be converted to dimethyl selenide, which can volatilize from the water to the atmosphere (reviewed in Lemly 2002; Chasteen and Bentley 2003).

If Se concentrations are lower in hydroelectric reservoirs, this may contribute to elevated Hg concentrations in muscle of fish in new reservoirs. Bioaccumulation of Hg is elevated in new hydroelectric reservoirs (Bodaly *et al.* 1984; Verdon *et al.* 1991; Bodaly and Fudge 1999; Hall *et al.* 2005); however, in one study Se was shown to lower methyl mercury (MeHg) bioaccumulation (Turner and Swick 1983). Mercury uptake by fish has been shown experimentally to be lower in the presence of elevated Se concentrations (Turner and Swick 1983). In a whole-lake study, Paulsson and Lundbergh (1989) found a 75% to 85% reduction in Hg concentrations of fish after Se additions to lakes in Sweden. In addition, Southworth *et al.* (1994; 2000) reported an increase in Hg concentrations in muscle of fish, and therefore a change in Hg bioavailability, following the elimination of Se-rich discharges from a coal-fired power plant into a quarry pit. Therefore if lower Se concentrations occur in new hydroelectric reservoirs, this may contribute to higher Hg concentrations in fish.

Consequently, Se additions have been considered to mitigate elevated Hg concentrations in fish. Although Se is an essential element and required for optimal physiological functions, it becomes toxic at narrowly elevated concentrations. Selenium easily transfers from female fish to their eggs and is then acquired by larvae from their yolk (Lemly 1998). Relatively low concentrations of Se can cause deformities to offspring or failure to recruit, without any signs of toxicity in adults. This occurs because excess Se can replace sulphur during protein synthesis, alter the structure of proteins, and alter the function of proteins (Lemly 1998). Therefore, it is unknown if Se can effectively lower Hg concentrations in fish without inducing Se toxicity. If Se is depleted in new reservoirs, there may be greater leeway for safely adding Se.

We assessed whether Se concentrations were lower in aquatic compartments of hydroelectric and experimental reservoirs than in natural lakes. In addition, we explored relationships between Hg or methyl mercury (MeHg) and Se concentrations. Our first null hypothesis was that flooding causes no effects on concentrations of Se in environmental compartments of hydroelectric reservoirs. Our second null hypothesis was that lowered

concentrations of Se in lakes and hydroelectric reservoirs do not affect Hg concentrations in the same compartments. If Se is lower in new reservoirs, these results could provide support for the addition of Se to reservoirs to restore background concentrations and lower Hg concentrations in fish. We expected to find lower Se concentrations in newer reservoirs because of higher methylation rates and lower oxygen concentrations, which are conditions that support the formation of volatile Se species (Chasteen and Bently 2003). Also, negative correlations between Hg and Se concentrations in environmental compartments may occur as correlations have been reported in other freshwater systems (Leskinen *et al.* 1986; Mauk and Brown 2001; Chen *et al.* 2001).

METHODS

Locations

In 2003 we sampled experimental reservoirs at the Experimental Lakes Area (ELA; 49°40' N; 93°43' W) in northwestern Ontario and hydroelectric reservoirs in northern Québec at La Grande Complex (53°45' N; 77°30' W) and Robertson Reservoir (59° N; 51° W). We compared results of reservoirs with reference lakes from the same region to account for geographic differences in Se concentrations. At the ELA, we compared samples from three experimental reservoirs located in an upland forest catchment area called FLUDEX (Flooded Upland Dynamics Experiment) 1, 2, and 3, one experimentally flooded wetland called the ELARP (Experimental Lakes Area Reservoir Project) reservoir, and four reference lakes. Lake 468 is the source of water for the FLUDEX reservoirs, and Lake 240 is the source of water for the ELARP reservoir. These reference lakes were chosen because they flowed directly into the experimental reservoirs, but water chemistry variables, chosen because they are known to affect Hg dynamics, were quite different among lakes (Table 1). The geography of the ELA is described in Brunskill and Schindler (1971). Further physical and chemical properties of the FLUDEX reservoirs are described in Hall et al. (2005) and those for the ELARP reservoir in Paterson *et al.* (1997). We also sampled two wetland ponds at the ELA, Lakes 632 and 115. These reference lakes were chosen because they contained the same fish species and were similar in size to the reservoirs. The FLUDEX reservoirs were in their fifth season of flooding, while the ELARP reservoir was in its twelfth year of flooding. Newly flooded reservoirs tend to be less oxygen-rich than natural lakes (reviewed in Bodaly *et al.* 1997), but we do not have oxygen data for systems in Québec. Some of the systems at ELA in our study had anoxic hypolimnia (Table 2), but it is possible that not all reservoirs thermally stratify. While the water

bodies studied at the ELA were relatively small, they approximate the processes of Hg dynamics in larger water bodies because most Hg methylation takes place in shallow waters and most aquatic organisms feed from these areas.

In northern Québec, we sampled two hydroelectric reservoirs of La Grand Complex-Laforge-1 Reservoir and Caniapisceau Reservoir-and Reference Lake Serigny (69°40'41"N; 55°20'59"W). These reservoirs were 20 years old. Robertson Reservoir in eastern Québec was eight years old and was compared with Reference Lake Ivry (58°57'27"N; 51°09'40"W). The reference lakes in Québec, pre-selected by Hydro-Québec as their long-term reference lakes for each hydroelectric reservoir, were used. Lakes were selected with fish populations that were large enough as to not be impacted by extensive sampling.

Collection of water, zooplankton, macro-invertebrates, fish, and soil samples

Dates

We collected samples of fish, macro-invertebrates (emerging insects at the ELA and Chironomid larvae in Québec), zooplankton, and water from each lake and reservoir, as well as flooded and unflooded soil from the reservoirs. Initial sampling at the ELA occurred during May 20 to 23 in Lakes 240, 468, 632, and 115, and the ELARP reservoir. The FLUDEX reservoirs were flooded in early June. The ELARP reservoir was re-flooded beginning on March 26. Fish were added to the FLUDEX and ELARP reservoirs on June 16. All systems at the ELA were sampled the weeks of June 16, July 28, and September 8, 2003. In the same year, sampling in La Grande Complex in northern Québec took place July 15 to 25 and August 6 to 12. Robertson Reservoir in eastern Québec was sampled for fish from June 16 to 23 and for all environmental compartments from August 18 to 29.

Water

We collected surface water samples for Se (100 mL), THg (125 mL), and MeHg (250 mL) analyses using trace-metal-clean techniques (St. Louis *et al.* 1994). Water samples for Se analyses were collected in high-density polyethylene bottles, whereas those for Hg analyses were collected in Teflon bottles. We acidified water samples for Se analyses with 0.5% ultra-high purity concentrated nitric acid (Fisherbrand) and refrigerated them. We acidified water samples for THg analysis with ultra-high purity concentrated hydrochloric acid (Baker) and water samples for MeHg analysis were frozen at -4°C until analysis, for four to eight weeks.

Macro-Invertebrates

At the ELA, we anchored floating emergent insect traps in shallow water in the experimental reservoirs and reference lakes. Insects were collected from traps on each sample date. Emerging insects were vacuumed from floating traps and frozen before transferring into bags. The traps covered 0.46 m^2 and the wood frame was covered with "no-see-um" mesh. In northern Québec we collected benthic invertebrates with a petit ponar dredge, as well as with 1 mm mesh D-ring nets. These samples were sieved and then benthic invertebrates were picked out using clean trays, tweezers, and pipettes. Zooplankton samples were collected using horizontal sweeps of a 150 μm mesh Wisconsin net attached to a wooden pole. The net contents were back-washed with lake water into bags. When systems were unproductive, oblique tows of a 60 cm diameter bongo net fitted with 150 μm mesh was used to collect zooplankton (Kelso and Rutherford 1996). Depending on the site, we used either a zodiac or an aluminium boat with a gas-powered outboard motor. All invertebrate samples were frozen at -4°C for four to eight weeks in polyethylene bags immediately after collection.

Fish

Fish from the ELA were collected using gill nets, minnow traps with bait canisters, hoop nets, and beach seine nets (Hayes *et al.* 1996). Finescale dace (*Phoxinus neogaeus*) were purchased from a local bait fisher to add to the reservoirs (Hall *et al.* 2005). At the time of collection, the fork lengths of finescale dace ranged from 52 to 92 mm. Finescale dace were not present in Reference Lakes 240 and 468. As a result, we sampled yellow perch (*Perca flavescens*; 52 to 109 mm) from these lakes. In Lake 468, we also collected pearl dace (*Margariscus margarita*; 49 to 118 mm). Comparisons between different species should be treated with caution, and as a result, we compared the same species of fish, finescale dace, from reservoirs and Reference Lakes 115 and 632 at the ELA. All fish from Québec were collected using gill nets. Fish species sampled from La Grande Complex were longnose sucker (*Catostomus catostomus*; 110 to 560 mm), lake whitefish (*Coregonus clupeaformis*; 254 to 560 mm), northern pike (*Esox lucius*; 215 to 975 mm), and lake trout (*Salvelinus namaycush*; 140 to 1040 mm). Fish species sampled from Robertson Reservoir and Reference Lake Ivry were rainbow smelt (*Osmerus mordax*; 120 to 252 mm), arctic char (*Salvelinus alpinus*; 125 to 570 mm), brook trout (*Salvelinus fontinalis*; 139 to 550 mm), and landlocked salmon (*Salmo salar ouananiche*; 136 to 520 mm). All fish were weighed to the nearest 0.1 g and fork length was measured to the nearest mm. In Québec, sub-samples of dorsal muscle were taken using a steel

knife. Fish from the ELA were weighed frozen and muscle from the dorsal region was sub-sampled for THg and Se. All sub-samples were frozen at -4°C for less than eight months in polyethylene bags.

Soil

The water depth was 20 cm where we collected flooded soil. Unflooded soil samples were collected near the reservoir above the maximum level of water. We collected soil samples using polycarbonate pipe with a bevelled edge and a rubber stopper. We pushed and twisted the pipe vertically into soil to about a 5 cm depth. For flooded soil, we applied a stopper to hold the contents by suction while we lifted the pipe. We lifted the pipe out of the substrate and inserted another rubber stopper in the bottom and then poured the water off the top. For unflooded soil samples we removed the material around the pipe and used a straight edge to cut the bottom of the soil core sample for removal. Soil samples were frozen at -4°C for less than eight months in polyethylene bags.

Analyses

Samples of soil, zooplankton, and invertebrates were freeze dried at -50°C. Soil samples were initially ground with an electric steel grinder. Before grinding, we washed the grinders with distilled water and Hg-clean wipes. We sent soil samples to our analytical laboratories (see below) in polyethylene bags. We weighed sub-samples of zooplankton, emerging insects, and Chironomid larvae on a Perkin Elmer microbalance (50 to 60 mg for THg and 10 to 20 mg for MeHg). We used an acid-rinsed stainless steel scoop to transfer the material into trace-metal-clean weigh boats. Sub-samples were placed in Teflon vials that had been washed in hot nitric acid. The vials were stored in polyethylene bags.

Analyses of Hg

Total Hg in fish from the ELA was analyzed by cold vapour atomic absorption spectrometry (CVAAS) at the Freshwater Institute where the Canadian Food Inspection Agency's Mercury Quality Assurance Program (MQAP) is conducted four times annually. Muscle of fish weighing 0.17 to 0.25 g (wet weight [w.w.]) was added to digestion tubes. Tissue was digested in 3 mL sulphuric acid and 2 mL nitric acid on an aluminium block at 180°C for five hours. At room temperature, $KHSO_4$ and $KMnO_4$ oxidants were added. Excess oxidant was reduced with a hydroxylamine sulphate/saline solution. The volume was increased

to 25 mL with deionized water. Total Hg was analyzed on a LDC Analytical Hg Monitor 3200. Standard CVAAS methods were modified from Armstrong and Uthe (1971) and Hendzel and Jamieson (1976). A Thermo Separation Products SP4400 Integrator digitally transformed peak areas. Blanks, Hg standards, and reference materials-Tort 2, Dolt, and Dorm-were analyzed in duplicate at the beginning and end of each run, as well as a previously characterized fish tissue from the MQAP. These analyses were always within expected ranges. Blanks were also analyzed mid-run. Samples were not analyzed in duplicate because of limited tissue.

Total Hg concentrations in samples of fish from Québec were also analyzed by CVAAS on a CETAC model M-6000A at the Philip Services Corporation Laboratory (now Maxxam Analytics) in Anjou, Québec. This laboratory also participated in the MQAP Samples of wet muscle of fish were digested following the protocol of Environment Canada's National Water Quality Databank (NAQUADAT) method No. 80601-2 (National Laboratory for Environmental Testing Manual of Analytical Methods, volume 2 trace metal 1994 methods 02-2601 and 02-2800). Each run analyzed seven standards of which five were certified. These analyses were always within expected ranges. Three blanks were analyzed, one without reagent, to monitor the THg concentration in the reagent. Standards and blanks were analyzed after every twentieth sample.

THg and MeHg in media from the ELA were analyzed by cold vapour atomic fluorescence spectrometry (CVAFS) by Flett Research Ltd. in Winnipeg, Manitoba. Water samples for THg analysis were prepared by the methods of Bloom and Fitzgerald (1988). To analyze MeHg in zooplankton, Chironomid larvae, and muscle of fish, 5 to 10 mg of homogenized sample were digested in 300 to 500 µg of KOH/MeOH overnight at 75°C on an aluminium hot block. Water samples for MeHg were distilled by the methods of Horvat *et al.* (1993). Forty-five mL of sample was distilled. An aliquot of 40 mL was acidified with KCl/H_2SO_4. For THg analysis of soil, about 0.1 g of dry sample was placed into acid-clean 20 by 150 mm test tubes and the exact weight of sample determined to 0.0001 g (Hendzel and Jamieson 1976). To all dry samples there was added 0.2 mL deionized water, and then 3 mL of acid (7:3 HNO_3 :H_2SO_4). The acid was at room temperature before an 18-hour digestion at 130°C in an aluminium hot block. When cooled, 200 µL of bromium chloride was added and the digests were brought up to 20 mL. The samples were then poured into 40 mL acid-cleaned EPA vials with a Teflon-lined cap. Aliquots of 0.1 to 1.0 mL were analyzed by CVAFS. Method detection limits were about 1 to 5 ng/g of Hg (dry weight [d.w.]) for soil. Spike and reference recoveries were typically 100 +/- 10%. For MeHg in soil, approximately 0.05 g of dry

sample was analyzed. In each batch of analysis, two samples were spiked at approximately 10 times the ambient concentration of MeHg in the samples. One mL of copper sulphate and 5 mL of acidic potassium bromide solutions were added. Then 10 ml of CH_2Cl_2 was added by weight. Soil samples were shaken for one hour and settled overnight. If the organic and aqueous layers were not separated, they were centrifuged for 20 minutes at the maximum rotation speed of 2000 RPM and 738 g of force. After separation, 2 mL of the methyl chloride layer was pipetted into a 60 mL Teflon distillation vial with 40 mL of deionized water. Once aliquoted, the samples were placed into a water bath at 50°C. Each sample was purged with clean nitrogen gas at approximately 45 mL/minute until all methyl chloride was volatilized. For low samples, about 20 to 40 mL was analyzed; for samples with high Hg concentrations, only 1 mL of 40 mL was analyzed. Then samples were treated as ordinary aqueous samples for analysis by ethylation, purging and trapping on Tenax, followed by thermal desorption onto a gas chromatographic column, where the separated species in the effluent were pyrolized and detected by CVAFS (Horvat et al. 1993; Liang *et al.* 1994; Logar *et al.* 2000; Logar *et al.* 2001).

Analyses of Se

Total Se in biota was analyzed by atomic absorption spectrometry in the Heavy Metals Laboratory at the Freshwater Institute in Winnipeg, Manitoba. Sub-samples of tissue (0.8 g [w.w.] for fish muscle and 0.1 to 0.3 g [d.w.] for invertebrates) and soil (0.4 g [d.w.]) were weighed into 25 x 200 mm Pyrex test tubes and digested with nitric (4 mL), sulfuric (0.5 mL) and perchloric (1 mL) acids for five hours at 130°C, followed by two hours at 200°C in a perchloric acid fume hood. Samples were cooled, and then 15 mL of distilled water and 7.5 mL of hydrochloric acid were added and heated for one hour at 90°C, cooled and adjusted to 25 mL with distilled water. Samples were analyzed by the automated addition of reductant and reagent blank on a Varian AA-55 attachment VGA-77 using Atomic Absorption Spectrometry. Blanks, calibration standards, and standard reference material were treated the same as the samples. The concentrations of Se in standards ranged from 0 to 25 µg/L. Duplicate analysis was done on every tenth sample and the coefficient of variation ranged from 0 to 14%.

Total Se concentrations in water were analyzed using inductively coupled plasma mass spectrometry (ICPMS) at the University of Manitoba Clean Laboratory. Five to 10 mL aliquots of final liquid extracts were analyzed. The detection limit was 0.02 µg/L. Certified natural water, selenite certified reference material, SLRS-4, and an internal standard, indium, were used to verify analytical precision.

Statistical Analyses

We compared the Se concentrations of each fish species, grouped across all sample dates, from each reservoir with its reference lake using paired t-tests. We compared finescale dace in the ELARP reservoir to finescale dace in Reference Lakes 115 and 632. These analyses allow assessment of within system variance relative to the differences among lakes. We also used t-tests to analyze differences between flooded and unflooded soil samples. Linear regression analysis tested the significance of the relationships between Hg or MeHg concentrations in tissues and water Se concentrations. All data were log-transformed to meet the assumptions for statistical analyses.

RESULTS

For each environmental compartment, we report the seasonal trend in Se concentrations, the range in Se concentrations, whether reservoirs had lower, similar, or higher concentrations of Se relative to reference lakes, and the significance of these relationships if statistics were used to analyze the given compartment.

In the second section of the results, we report relationships between Hg or MeHg and Se concentrations in each of the environmental compartments, the details and significance of correlations, and whether correlations supported the null hypothesis that Se did not affect Hg or MeHg cycling in aquatic food webs. For each section we followed this sequence of reporting: ELARP, FLUDEX, La Grande Complex, and Robertson reservoirs. Concentrations in muscle of fish are reported in wet weight and all other tissues including soil are reported in dry weight.

Were Se concentrations depressed in reservoirs relative to lakes?

Fish

Concentrations of Se in muscle of fish from the ELARP reservoir and associated reference lakes were fairly consistent throughout the summer. Concentrations of Se in muscle of fish ranged from 0.2 to 0.6 μg/g in reference lakes and were less than 0.2 μg/g in the ELARP reservoir (Figure 1). Concentrations of TSe in muscle of finescale dace in the ELARP reservoir were significantly lower than those in Reference Lakes 115 and 632 ($p<0.0001$ and $p=0.0003$, respectively; Table 3). The data support our alternative hypothesis that Se concentrations were lower in fish in this reservoir.

Seasonal trends in Se concentrations in muscle of fish were inconsistent among the FLUDEX reservoirs. The reference lakes for FLUDEX, however, did not noticeably change during the summer. We compared finescale dace in the FLUDEX reservoirs with finescale dace from Lakes 115 and 632 (Figure 1). Finescale dace from reference lakes had mean Se concentrations between 0.2 and 0.75 μg/g. Concentrations of Se in the muscle of finescale dace from the FLUDEX reservoirs ranged from 0.2 to 0.6 μg/g. In August, concentrations of Se in muscle of fish from FLUDEX reservoirs 1 and 2 were significantly lower than those in Reference Lake 115 ($p=0.0015$ and $p=0.0081$, respectively; Table 3; Figure 1), while concentrations of TSe in muscle of fish from FLUDEX reservoir 2 and 3 were significantly higher than those in Reference Lake 632 ($p=0.023$ and $p<0.0001$, respectively). Concentrations of Se were not consistently lower than those in Reference Lakes 115 and 632, but they were consistently lower than those in pearl dace from Reference Lake 468.

Large fish from Québec were sampled throughout the summer and had mean concentrations of Se in muscle that ranged from 0.2 to 0.6 μg/g (Figure 2). These concentrations are similar to those in small fish from the ELA. Longnose sucker ($p=0.0006$), lake whitefish ($p=0.0003$), and northern pike ($p<0.0001$) had lower Se concentrations in Caniapisceau Reservoir than in the reference lakes. Lake trout ($p=0.008$) had significantly higher Se concentrations in muscle than those in the reference lake. In Laforge-1 Reservoir, longnose sucker, lake whitefish, and northern pike had significantly lower Se concentrations in muscle than those in reference lakes ($p<0.0001$, 0.0033, and $p<0.0001$, respectively). All of this data except the lake trout support the null hypothesis that Se concentrations were lower in reservoirs relative to reference lakes.

In Robertson Reservoir and its reference lake, Se concentrations in muscle of fish ranged from 0.3 to 0.7 μg/g (Figure 2). Rainbow smelt ($p=0.0036$) and brook trout ($p=0.0012$) from Robertson Reservoir had lower Se concentrations in muscle compared to those in Reference Lake Ivry. Arctic char from Robertson Reservoir had higher Se concentrations than those in Reference Lake Ivry ($p=0.0467$). Concentrations of Se in muscle of landlocked salmon from Robertson Reservoir were not statistically different from those in Reference Lake Ivry. Rainbow smelt and brook trout data supported the hypothesis that Se was depressed in this reservoir. Results from arctic char and landlocked salmon did not support the hypothesis.

Zooplankton

In the ELARP reservoir, Se concentrations in zooplankton decreased during the summer while they increased in Reference Lake 240 (Figure 3). Concentrations of Se in zooplankton ranged from 0.5 to 1 μg/g in the ELARP reservoir and 0.5 to 3.5 μg/g in Reference Lake 240. Zooplankton in the reference lake had two to threefold higher Se concentrations than in the ELARP reservoir in July and September, but not in June. Data from July and September suggested there were lower Se concentrations in zooplankton in the ELARP reservoir as a result of flooding, although the data in June contradicted this.

Through June, July, and September, Se concentrations in zooplankton from FLUDEX reservoirs 2 and 3 and Reference Lake 468 had an overall increasing trend characterized by a mid-summer peak (Figure 3). Concentrations of Se in zooplankton in FLUDEX reservoir 1 increased throughout the season. Concentrations of Se in zooplankton in FLUDEX reservoirs ranged from 0.1 to 2 μg/g while those in Reference Lake 468 ranged from 1.3 to 5 μg/g. Concentrations of Se in zooplankton in FLUDEX reservoirs were consistently lower than those in Reference Lake 468. These data suggest Se concentrations were lower in zooplankton because of flooding. Note that concentrations of Se in zooplankton in the ELARP reservoir and Reference Lake 468 exceeded the toxicity threshold for Se in the diet of fish.

In La Grande Complex, Se concentrations in zooplankton increased from July to August in the reservoirs, while it decreased in Reference Lake Serigny (Figure 4). Concentrations ranged from 0.3 to 1.5 μg/g of Se. Reference Lake Serigny had lower Se concentrations in zooplankton than those in Caniapisceau Reservoir and more than those in Laforge-1 Reservoir. There were no consistent differences between Se concentrations in La Grande complex reservoirs and their reference lake.

During the summer, Se concentrations in zooplankton from Robertson Reservoir and Reference Lake Ivry increased (Figure 4). They ranged from 0.5 to 2.8 μg/g of Se. In June, zooplankton in this reservoir contained higher Se concentrations than those in the reference lake, but this relationship was reversed in August. Again, the evidence for Se depression in zooplankton in reservoirs was inconsistent.

Insects

Concentrations of Se in emerging insects decreased seasonally in the ELARP reservoir and Reference Lake 240, were constant in Reference Lake 632, and increased in Reference Lake 115 (Figure 5). Concentrations of Se in emerging insects in the ELARP reservoir ranged from

0.5 to 4 µg/g. They were consistently about four times less than those in Reference Lake 240, were also lower than those in Reference Lake 115, but were similar to those in Reference Lake 632. Because Reference Lake 240 flowed directly into the ELARP Reservoir and was therefore a direct comparison, these data support the rejection of our null hypothesis.

Consistent concentrations of Se were measured in emerging insects in FLUDEX reservoirs 2 and 3 and Reference Lake 632 throughout our sampling season (Figure 5). Concentrations of Se increased in Reference Lake 115 and decreased in FLUDEX reservoir 1 from July to September. In June there was not enough mass of emerging insects from any FLUDEX reservoir, likely due to predation by spiders in the traps. Also, in Reference Lake 468 there was insufficient biomass to analyze Se concentrations. Concentrations of Se in these systems ranged from 0.5 to 1.8 µg/g, except in Reference Lake 115 in September, which contained 2.8 µg/g. In July, Se concentrations in emerging insects in the FLUDEX reservoirs were similar to Lakes 115 and 632. In September, Reference Lake 115 contained two times higher Se concentrations than those in the FLUDEX reservoirs and Reference Lake 632. There was therefore support for both higher and lower Se concentrations in emerging insects from these systems.

In La Grande Complex, Se concentrations in Chironomid larvae were similar on each sample date in Reference Lake Serigny and Laforge-1; however, Caniapisceau Reservoir concentrations decreased noticeably through the season (Figure 6). We acquired Chironomid larvae samples from Robertson Reservoir only in August and therefore we cannot report seasonal trends. Concentrations of Se in Chironomid larvae in La Grande Complex and Robertson Reservoir ranged from 0.5 to 1.5 µg/g, except for those in Caniapisceau Reservoir in July, which contained 9 µg/g of Se. Concentrations of Se in Chironomid larvae in August from the two areas of Québec were higher in reference lakes than in reservoirs. Concentrations of Se in Chironomid larvae in La Grande Complex in July did not differ from those in Reference Lake Serigny. Concentrations of Se in Chironomid larvae in Caniapisceau Reservoir were eight times greater than those in Reference Lake Serigny. These data both supported and contradicted our null hypothesis.

Water

In the ELARP reservoir and its reference lake, Se concentrations in whole water decreased from June to July and then increased (Figure 7). There was an overall decreasing trend with mid-summer minima. Concentrations of Se in water ranged from 0.005 to 0.05 µg/L

in the reservoir and 0.08 to 0.15 µg/L in the reference lake. Concentrations of Se in surface water in the ELARP reservoir were consistently lower than those in Reference Lake 240, which contradicts our null hypothesis that Se concentrations were not depressed.

Seasonal trends of Se concentrations in whole water from the FLUDEX reservoir and Reference Lake 468 were dissimilar. The concentrations of Se in water ranged from 0.03 to 0.20 µg/L (Figure 7). In June, concentrations of Se in Reference Lake 468 were below detection and we assumed this measurement to be an error because they were outside of all other measurements. Only Se concentrations in water in FLUDEX 3 in July were lower than Se concentrations in water in the reference lake. Therefore, it appears Se concentrations in water were not lower due to flooding these reservoirs.

Throughout the season, concentrations of Se in water varied in La Grande Complex. We analyzed samples of water from Robertson Reservoir in August. Water samples collected in June could not be analyzed. Concentrations of Se increased in water in Laforge-1 Reservoir and in Reference Lake Serigny while they decreased in Caniapisceau Reservoir. Concentrations of Se in water ranged from 0.02 to 0.06 µg/L (Figure 8). Water in Laforge-1 Reservoir had consistently lower Se concentrations than those in its reference lake. In August Caniapisceau Reservoir also had lower Se concentrations in water than those in its reference lake, but in July it was higher. Robertson Reservoir had greater concentrations of Se in water than those in its reference lake. These results were mixed and therefore this test of our hypothesis was inconclusive.

Soil

Flooded soils had the same or higher mean Se concentrations than unflooded soil. Concentrations ranged from 0.2 to 0.5 µg/g (Figure 9). Concentrations of Se in flooded and unflooded soil in each reservoir were not significantly different (ELARP: $p=0.76$; FLUDEX 1: $p=0.34$; Caniapisceau: $p=0.98$; Robertson: $p=0.57$) except in FLUDEX 3 ($p=0.05$). These data support our null hypothesis that Se concentrations were not depressed in soil or reservoirs.

Were there relationships between THg or MeHg and Se concentrations in environmental compartments?

Fish

When all systems were grouped, there were lower Hg concentrations in muscle of fish when higher Se concentrations in muscle of finescale dace at the ELA (Figure 10). There were

no releationships when each system was analyzed separately. Concentrations of Hg in muscle of fish ranged from 0.03 to 0.45 μg/g. Hg concentrations in muscle were not significantly related to Se concentrations in muscle of fish.

When all species from La Grande Complex were grouped, Hg concentrations in muscle were lower when Se concentrations in muscle were low, while high Se concentrations in muscle corresponded with both low and high Hg concentrations in muscle (Figure 11). Concentrations of Hg in muscle ranged from 0.05 to 6 μg/g in most fish. A few fish contained 7 to 10 μg/g of Hg in muscle. Concentrations of Hg in muscle were 0.1 to 6 μg/g. Hg concentrations in muscle were not significantly related to Se concentrations in muscle of fish.

Fish from Robertson Reservoir also showed lower Hg concentrations in muscle when Se concentrations in muscle were low and low and high Hg concentrations in muscle when Se concentrations in muscle were higher (Figure 11). Concentrations of Hg in muscle ranged from 0.1 to 5 μg/g. This relationship between Hg and Se concentrations in muscle was not significant and therefore these data suggested that Se concentrations did not affect Hg concentrations in muscle of fish in Robertson Reservoir.

Zooplankton

There was an inverse relationship between MeHg and Se concentrations in zooplankton at the ELA (Figure 12). Concentrations of MeHg in zooplankton usually ranged from 25 to 200 ng/g. One sample of zooplankton contained 550 ng/g of MeHg. Concentrations of Se usually ranged from 0 to 4 μg/g. The relationship between MeHg and Se concentrations was not significant ($p=0.15$; $r^2=0.31$). We failed to reject our null hypothesis that Se concentrations did not affect MeHg concentrations in zooplankton.

We grouped zooplankton data from the two regions in Québec to analyze Hg and Se relationships (Figure 12). There was no relationship between MeHg and Se concentrations in zooplankton in lakes and reservoirs in Québec ($r^2=0.09$; $p=0.61$). Zooplankton in Québec had concentrations of MeHg from 50 to 275 ng/g and Se concentrations from 0.25 to 2.75 μg/g.

Insects

Concentrations of MeHg in emerging insects usually ranged from 0 to 100 ng/g and Se concentrations usually ranged from 0 to 2 μg/g (Figure 13). One sample had high concentrations of both MeHg and Se (450 ng/g and 4 μg/g, respectively). Including all emerging insect data from the ELA, there was a significant positive relationship between MeHg

and Se concentrations (r^2=0.82; p=0.005; log(y)=1.75+1.26*log(x), where x is the Hg concentration in Chironomid larvae and y is the Se concentration in water). This relationship was due to one sample, which when removed was not significant (r^2=0.59; p=0.08). These data do not support rejecting our null hypothesis that Se affected MeHg concentrations in emerging insects.

There was no relationship between MeHg and Se concentrations in Chironomid larvae from the Québec lakes and reservoirs (r^2=0.24; p=0.40; Figure 13). Concentrations of MeHg ranged from 90 to 200 ng/g while Se concentrations ranged from 0.5 to 1.5 µg/g. Almost all of the Chironomid larvae collected in Québec had very low Se concentrations.

Water

In water at the ELA there was no linear relationship between Hg and Se concentrations (Figure 14). In water, concentrations of THg ranged from 1 to 12 ng/g while concentrations of Se ranged from 0.05 to 0.12 µg/g. Concentrations of THg in water increased with increasing concentrations of Se, but this relationship was not significant (r^2=0.26; p=0.20).

Concentrations of MeHg in water at the ELA were also not related to concentrations of Se in water (Figure 14). In water, MeHg concentrations ranged from 0 to 0.8 ng/L and Se concentrations ranged from 0.05 to 0.12 µg/L. Because there was no significant relationship, these data suggest that Se concentrations did not affect MeHg concentrations in water at the ELA (r^2=0.11; p=0.41).

There was no relationship between Hg and Se concentrations in water in Québec (r^2=0.11; p=0.58; Figure 15). Concentrations of MeHg ranged from 1.5 to 2.8 ng/L and Se concentrations ranged from 0.01 to 0.06 µg/L. We failed to reject our null hypothesis that Se concentrations did not affect Hg concentrations in surface water in Québec.

There was no relationship between concentrations of MeHg and Se in water in Québec (r^2=0.03; p=0.78; Figure 15). The range in MeHg concentrations was 0.02 to 0.19 ng/L, while Se concentrations ranged from 0.01 to 0.06 µg/L. We failed to reject the hypothesis that Se concentrations do not affect MeHg concentrations in these waters.

Soil

In flooded soil Hg and MeHg concentrations had no relationship with Se concentrations (Figure 16). It was noticeable that Hg and MeHg concentrations increased with Se concentrations in soil and then decreased. The greatest Hg and MeHg concentrations were in

soil that contained 0.4 μg/g of Se. Soil with 0.5 μg/g of Se had lower Hg and MeHg concentrations. Concentrations of Hg ranged from 30 to 120 ng/g and MeHg concentrations ranged from 1 to 14 ng/g. Concentrations of Se ranged from 0.2 to 0.5 μg/g. For these five reservoirs there was not a significant relationship between Hg or MeHg concentrations and Se concentrations in flooded soil (Hg: r^2=0.01; p=0.87; MeHg: r^2=0.41; p=0.24). We failed to reject the hypothesis that Se concentrations did not affect Hg or MeHg concentrations in these soils.

DISCUSSION

Se was not consistently lower in reservoirs relative to reference lakes

We found support for the hypothesis that Se concentrations are lower in reservoirs relative to reference lakes in less than half of the sampled compartments (Table 4). The remaining compartments had Se concentrations that were the same or higher in reservoirs relative to reference lakes and in these cases we failed to reject our null hypothesis: Se concentrations were not consistently depressed in reservoirs. Compartments from the ELARP and Laforge-1 reservoirs had the most support for depressed Se concentrations. Compartments in the FLUDEX reservoirs had variable Se concentrations. In some compartments, Se concentrations were higher in Caniapisceau and Robertson reservoirs than in their reference lakes. Concentrations of Se were depressed in muscle of fish from the ELARP reservoir, FLUDEX reservoirs 1 and 2, all fish from La Grande Complex except lake trout, and in rainbow smelt and brook trout from Robertson Reservoir. The remaining species from Québec and those from FLUDEX reservoirs contained Se concentrations similar to or greater than those in their reference lakes.

The reservoirs sampled at the ELA contained finescale dace that were transplanted from a nearby lake. Concentrations of Se in these fish may not have been at steady state with these systems. The half-life of Se in small fish may be 20 to 30 days (reviewed in Hamilton 2004). It seems reasonable that transplanted fish would represent Se dynamics of a system after acclimatizing for a season. Also, it is reasonable they would change notably between monthly sampling. Thus we believe that fish transplanted from a natural lake to a reservoir would reflect seasonal changes in the system and also whether Se concentrations were increasing, decreasing, or remaining unchanged.

Concentrations of Se in zooplankton from the ELARP, FLUDEX, and Laforge-1 reservoirs were lower than their reference lakes and may indicate that Se was depressed.

Zooplankton from Robertson Reservoir had temporally shifting Se concentrations whereas zooplankton from Caniapisceau Reservoir had higher Se concentrations than those in its reference lake. There was support for Se depression in the water of the ELARP reservoir that was directly fed by Reference Lake 240. Data from Laforge-1 Reservoir also supported the hypothesis of Se depression in water, though the reference lake did not flow directly into this reservoir. Waters in Caniapisceau and Robertson reservoirs also were not fed by reference lakes and we failed to reject our null hypothesis. Support for the hypothesis of Se depression in these reservoirs varied.

There were similarities between Se concentrations measured in our study and those in the literature. Concentrations of Se found in fish were similar to those in other studies of uncontaminated systems. Concentrations of Se in benthic invertebrates ranged 1.3 to 11 μg/g (d.w.) in a contaminated lake in Australia (Peters *et al.* 1999a), which coincides with the range we measured. We found high Se concentrations in soil relative to a study in Kansas (0.09 μg/g [d.w.]; Allen and Wilson 1990). Other studies observed background Se concentrations in water similar to those we measured (0.09 to 0.73 μg/L in Sudbury; Chen *et al.* 2001; 0.4 to 1.0 μg/L in Kansas; Allen and Wilson 1990).

Hg or MeHg and Se concentrations correlated in emerging insects and fish

We tested whether Se concentrations in the given environmental compartments were correlated with their Hg or MeHg concentrations. We rejected our null hypothesis that Se and Hg concentrations were consistently correlated in fish from the ELA and La Grande Complex. Hg concentrations in muscle of fish were lower when Se concentrations were higher in muscle of fish at the ELA. The contradicting relationships between Hg and Se in muscle of fish from these different regions may be due to fish species, size, or trophic level. In addition, other water chemistry characteristics could affect the Hg concentrations in the fish. For all other compartments, we failed to reject our null hypothesis that Se concentrations did not affect Hg concentrations.

In muscle of fish from Québec, Hg and Se concentrations occurred as follows: we measured only low Hg concentrations when Se concentrations were low and low to high Hg concentrations at relatively higher Se concentrations. It seemed that when Se concentrations were high, it was also possible for fish to contain higher Hg concentrations. This relationship is contrary to other studies that showed negative relationships between Hg and Se concentrations in fish (Leskinen *et al.* 1986; Mauk and Brown 2001; Chen *et al.* 2001). This contradiction in

the relationship between Hg and Se in fish may indicate that the main mode of action may be external to fish.

Elevated Se concentrations in muscle of fish did not appear to limit Hg concentrations. Fish in older reservoirs have a long-term integration of Hg. Turnover of Hg and Se in muscle of fish may take months to years (Van Walleghem *et al.* 2007; reviewed by Hamilton 2004). Larger and older fish seem to retain Se and Hg burdens. Larger fish have longer half-lives of Se (bluegill: 60 d; razorback sucker: 100 d; Colorado pikeminnow: >3 years). One reason for slower depuration of Se is that its organic form, selenomethionine, associates with protein.

The negative relationship between MeHg and Se concentrations in zooplankton from the ELA may indicate that Hg and Se interacted in the lower food web. For example, a negative relationship between Hg and Se concentrations was also found in Swedish systems (H. Hultberg, pers. comm. IVL, Sweden). Also, in a mesocosm study, radio-labeled Hg did not enter the lower food web when 100 µg/L of Se was added to water (Rudd *et al.* 1980). However, Se did not affect Hg concentrations in the lower food web organisms of a subsequent experiment by the same group of scientists (Turner and Rudd 1983). Because invertebrates may acquire MeHg and Se directly from water as well as from their food (Luoma *et al.* 1992; Canton and Van Derveer 1997), this relationship in zooplankton could reflect an interaction that took place in the water. No relationship between Hg or MeHg concentrations and Se concentrations were seen in water. Water chemistry can have an effect on Se concentrations in compartments that could distort differences in Se concentrations between reservoirs and reference lakes. We conclude that Se modified MeHg uptake or retention in zooplankton from the ELA, but we cannot explain why this occurred or why this relationship was not seen in zooplankton in Québec. Probably this relationship was not observed in Québec because we collected samples from fewer systems and therefore had fewer comparisons. Also, La Grande Complex was 20 years old whereas the ELA systems were five to 12 years old.

Factors influencing Se concentrations in reservoirs and reference lakes

Our data showed that differences in Se concentrations in various environmental compartments in reservoirs compared to reference lakes were not consistent, or that Se is not affected by reservoir creation. Differences between reservoirs and reference lakes may have been due to the similarity and proximity of reference lakes to reservoirs, reservoir age, water chemistry such as redox state and metal content, fish age, growth rate, diet, metabolism, spawning, and migration. Water that entered the ELARP and FLUDEX reservoirs came directly

from their respective reference lakes. The ELARP reservoir had lower Se concentrations in all environmental compartments except soil while the FLUDEX Reservoirs had variable to lower Se concentrations. Part of the reason for variability in the FLUDEX reservoirs may have been because of short turnover times in the FLUDEX reservoirs, and thus there was little opportunity for Se to decrease. Reservoirs in Québec were far from reference lakes. Reference lakes were selected to have many similar characteristics of reservoirs, but not necessarily close proximity. These reference lakes did not flow into reservoirs and were smaller than the reservoirs. We expect that the reservoirs had greater degrees of anoxia than reference lakes (Table 2). Selenium, as selenide or elemental Se, may bind with metals and sulphides in anoxic conditions (Naveau *et al.* 2007) whereas oxide forms of Se, selenite and selenate, are more mobile (Masscheleyn *et al.* 1990; Peters *et al.* 1999). Reservoir age may also have caused differences. Newer reservoirs would have had more anoxia, methylation, and higher Hg concentrations. Older reservoirs may have equilibrated to ambient processes and have been more comparable to reference lakes. In addition, algal community composition affects the concentrations of Se at the base of the food web (Baines and Fisher 2001) as does biomass of bacteria and phytoplankton (Baines *et al.* 2004).

Factors specific to fish may also have been responsible for differences between lakes and reservoirs. The prey eaten or the length of the food web may have varied from lake to lake as well as regionally. Although Se bioaccumulates, it does not always biomagnify, and therefore older fish or top predators may have lower Se concentrations than their prey (Besser *et al.* 1993). Metabolism of fish may be related to local climate or seasonal temperatures, while the productivity of a lake may affect the growth rate of fish. When fish grow faster, the amount of metals acquired may be diluted in their mass and be reflected as lower concentrations (Larsson *et al.* 1992). In addition, spawning can cause gender differences in Se concentrations as Se transfers to eggs and therefore decreases in females (Lemly 2002). Therefore the time of sampling relative to spawning could influence results.

Factors influencing regional differences in Se concentrations between the ELA and Québec

Selenium concentrations in water, macro-invertebrates, and zooplankton in Québec were generally less than half of those at the ELA. Here we summarize our findings between the two areas. The fish sampled from the ELA and northern Québec were all between 0.2 and 0.6 μg/g of Se. Zooplankton concentrations in the ELA were 1 to 5 μg/g while those in Québec were 0 to 2.5 μg/g. Emerging insects ranged from 0 to 4 μg/g in the ELA and Chironomid larvae ranged

from 0 to 0.15 μg/g in Québec (one sample contained 8 μg/g). Water from the ELA systems contained 0 to 0.2 μg/L and water from the Québec systems contained 0.02 to 0.06 μg/L of Se.

Possible explanations for the regional differences in Se concentrations are: bedrock, pollution, agriculture, glaciations, water turn-over, species of fish, age and size of fish, productivity, and temperature. Regional differences in Se concentrations are natural. Some soils are naturally enriched or depleted depending on the composition of the parent rock from which they formed. Also, Se may be elevated in some areas due to non-point-source emission, such as fossil fuel combustion or directly from coal or metal mining and smelting, as at Sudbury, Ontario (Chen *et al.* 2001). Concentrations of Se in soil establish regional background variations. In addition, biological processes may impose regional differences. The fish at the ELA were much smaller than those caught in Québec. Emerging insects from the ELA are not really comparable with Chironomid larvae in Québec because these may be different species with differring physiology, biodynamics, and life stages.

One consideration is that only total Se was analyzed. Analyzing Se species in future studies may garner more information about the mechanisms of Se cycling in freshwater systems. In aquatic organisms, Se is mostly present as organic Se (Fan *et al.* 2002; R.J. Flett, pers. comm., Winnipeg, Manitoba) and therefore analyzing total Se is probably accurate. Comparisons could not be made between flooded systems in different regions. In addition, analyzing MeHg in small fish would also be valuable as Se may affect THg and MeHg differently in muscle of young fish (Belzile *et al.* 2006). In concluding, Se concentrations were not consistently lower in reservoirs relative to reference lakes.

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Table 1. Water chemistry variables measured from the epilimnion in a selected location of each water body. Each value is the mean of all samples collected with one standard deviation (1 SD).

	pH	1 SD	DOC (µmol/L)	1 SD	Chlorophyl-a (µg/L)	1 SD	SO_4 (mg/L)	1 SD
Reference Lake 115	6.8	0.2	1013.0	9.9	2.8	0.8	1.8	0.0
Reference Lake 240	7.2		574.8	16.9	1.5	0.4	2.4	
Reference Lake 468	7.4	0.0	426.5	4.9	1.0	0.1	2.3	0.2
Reference Lake 632	6.5	0.0	1031.5	7.8	3.4	2.4	1.4	0.0
ELARP reservoir	5.9	0.1	1290.8	168.9	2.5	1.0	0.9	0.3
FLUDEX 1 reservoir	6.3	0.1	621.2	50.6	5.1	3.3	1.7	0.1
FLUDEX 2 reservoir	6.4	0.1	580.4	54.1	2.1	0.6	2.0	0.1
FLUDEX 3 reservoir	6.6	0.1	503.6	27.1	1.7	0.3	2.2	0.1
Reference Lake Serigny	6.7		356.0		0.7		0.5	
Caniapisceau Reservoir	6.9		276.0		1.2		0.7	
Laforge-1 Reservoir	6.7		516.5		2.4		0.3	
Reference Lake Ivry	6.3		448.0		0.9		0.9	
Robertson Reservoir	6.5		394.0		3.8		0.9	

Table 2. Profiles of oxygen (mg/L) in water bodies at the ELA in June, July, and September. Data for FLUDEX reservoirs are given as surface, middle, and bottom depths.

JUNE

Depth (cm)	Lake 468	Lake 240	Lake 115	Lake 632	ELARP reservoir	FLUDEX 1	FLUDEX 2	FLUDEX 3
0	8.0	8.0	7.7	7.5	4.4	6.6	6.6	7.1
20	8.4	7.9	7.6	7.3	4.4	6.2	6.3	6.9
40	8.3	8.0	7.7	7.3	4.0	4.9	5.1	7.0
60	8.4	8.1	7.3	7.8	3.1			
80	8.3	8.0	5.0	5.4	0.1			
100	8.2	8.0			0.1			
120	8.5	8.0			0.1			
140	8.0	8.0			0.1			
160	5.8	8.0			0.1			
180		8.0			0.1			
200		8.0			0.1			

JULY

Depth (cm)	Lake 468	Lake 240	Lake 115	Lake 632	ELARP reservoir	FLUDEX 1	FLUDEX 2	FLUDEX 3
0	7.9	7.8	7.5	6.6	3.7	6.0	6.4	6.6
20	7.9	7.7	7.1	6.5	3.3	5.8	6.1	6.4
40	8.0	7.8	7.7	6.7	2.5	5.4	5.2	6.2
60	8.2	6.9		6.5	2.2			
80	7.9	7.4		4.7	2.1			
100	8.1	7.7			<2.0			
120	8.0	7.4						
140	8.2	7.0						
160		7.3						
180		7.3						
200		7.7						

SEPTEMBER

Depth (cm)	Lake 468	Lake 240	Lake 115	Lake 632	ELARP reservoir	FLUDEX 1	FLUDEX 2	FLUDEX 3
0	8.1	8.2	8.0	7.7	3.7	6.0	5.7	7.0
20	8.1	8.3	7.7	7.1	3.6	5.7	5.5	6.8
40	7.6	8.2	7.7	7.0	3.5	5.5	5.4	6.5
60	8.0	8.2	7.4	6.9	3.6			
80	8.2	8.3	7.6	6.4	3.5			
100	7.9	8.3	<2.0	5.4	3.4			
120	8.1	8.2			3.6			
140	8.1	8.3			3.6			
160	7.7	8.2			3.1			
180	7.5	8.3			<2.0			
200		8.3						

Table 3. Results of ANOVA statistical tests of Se concentrations in muscle of fish where n are the sample sizes and the level of signifiance is 0.05. The arrow beside the P-Value indicates whether the concentration of Se in the muscle of fish from the reservoir was higher or lower than that in the reference lake.

Reference Lake (n)	**Reservoir (n)**	**Species**	**F-Values**	**P-Values**
Reference Lake 115 (30)	ELARP (19)	Finescale dace	85.29	<0.0001 ↓
	FLUDEX 1 (20)	Finescale dace	11.32	0.0015 ↓
	FLUDEX 2 (21)	Finescale dace	7.61	0.0081 ↓
	FLUDEX 3 (19)	Finescale dace	0.84	0.3645
Reference Lake 632 (30)	ELARP reservoir (19)	Finescale dace	15.47	0.0003 ↓
	FLUDEX 1 (20)	Finescale dace	3.45	0.0692
	FLUDEX 2 (21)	Finescale dace	5.51	0.023 ↑
	FLUDEX 3 (19)	Finescale dace	19.65	<0.0001 ↑
Reference Lake Serigny	Caniapisceau Reservoir			
(25)	(31)	Longnose sucker	13.38	0.0006 ↓
(26)	(28)	Lake whitefish	15.46	0.0003 ↓
(8)	(32)	Northern pike	188.59	<0.0001 ↓
(32)	(39)	Lake trout	7.44	0.008 ↑
Reference Lake Serigny	Laforge-1 Reservoir			
(25)	(36)	Longnose sucker	32.24	<0.0001 ↓
(26)	(27)	Lake whitefish	9.48	0.0033 ↓
(8)	(31)	Northern pike	117.98	<0.0001 ↓
Reference Lake Ivry	Robertson Reservoir			
(5)	(43)	Rainbow Smelt	9.43	0.0036↓
(28)	(52)	Brook Trout	11.24	0.0012 ↓
(31)	(29)	Arctic Char Landlocked salmon	4.13	0.0467 ↑

Table 4. Summary of results indicating whether Se concentrations were lower, the same, or higher in reservoirs relative to their references in each environmental compartment sampled. Soil samples from Laforge-1 and FLUDEX 1 were not analyzed.

	ELARP	FLUDEX	Caniapisceau	Laforge-1	Robertson
Water	↓	↕	↓	↓	↑
Zooplankon	↓	↓	↑	↕	↕
Insects	↓	↕	↕	↓	↓
Fish	↓	↕	↕	↓	↕
Soil	-	-	-		-

FIGURES

Figure 1. Concentrations of Se in muscle of fish from the ELA. Each bar is the mean concentration of ten fish with one standard deviation.

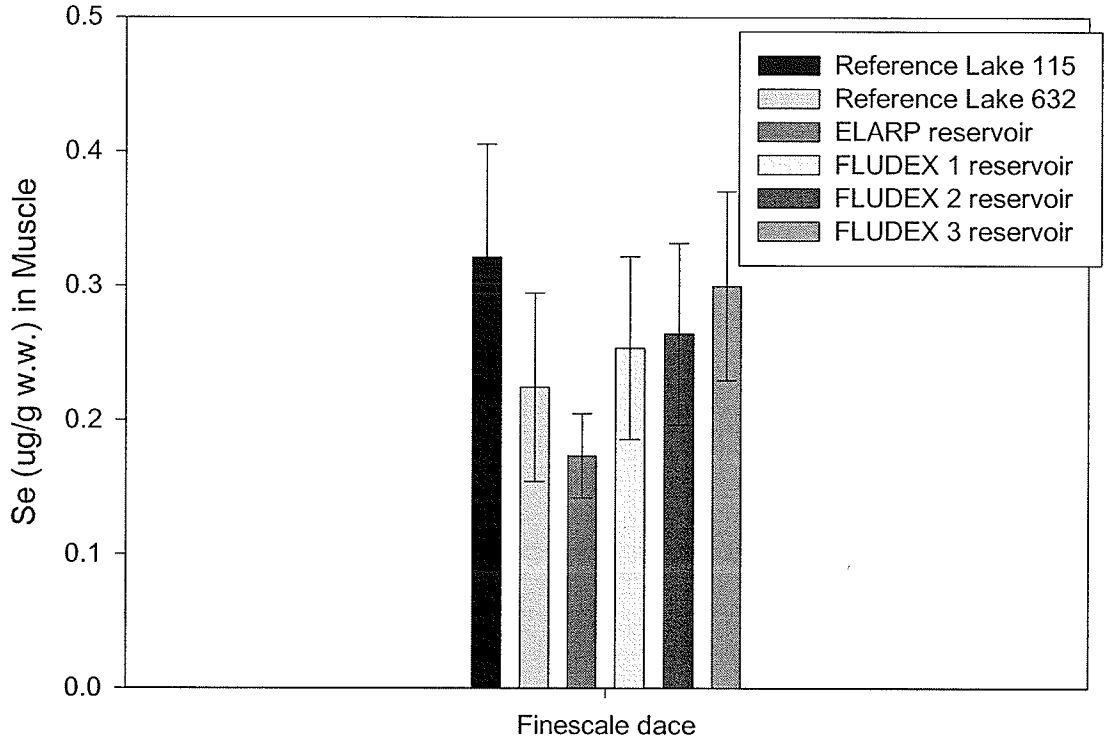
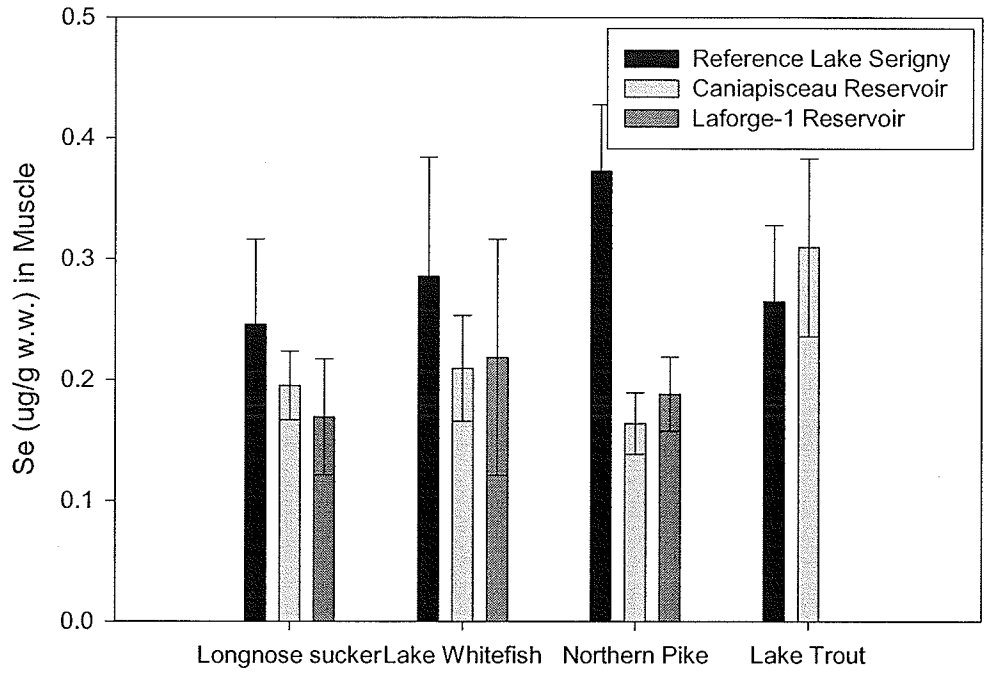


Figure 2. Concentrations of Se in muscle of fish from La Grand Complex and Robertson Reservoir. Each bar is the mean concentration of more than ten fish with one standard deviaiton.



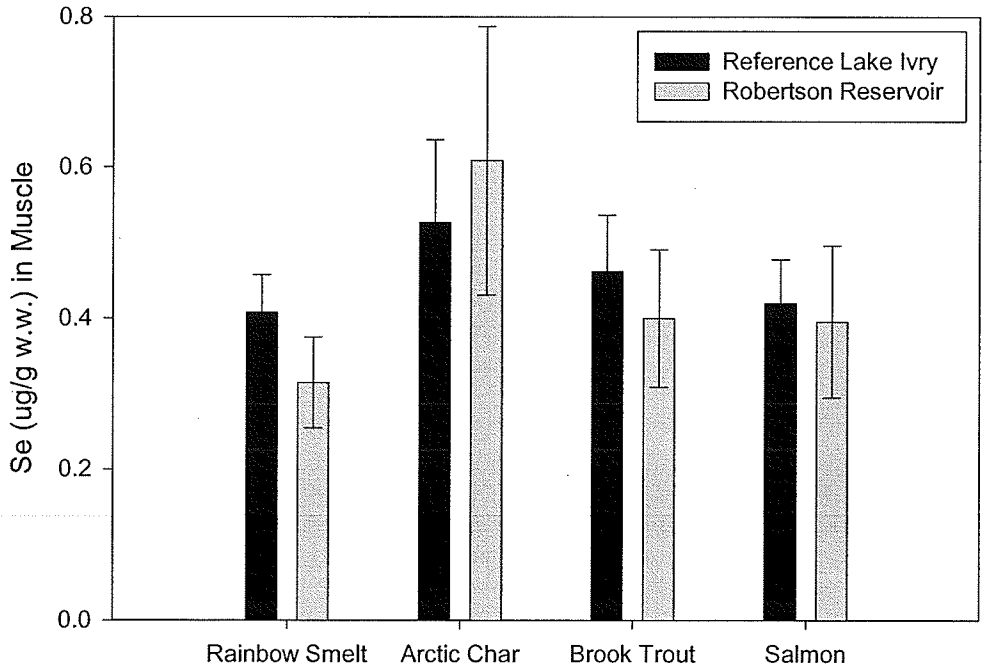
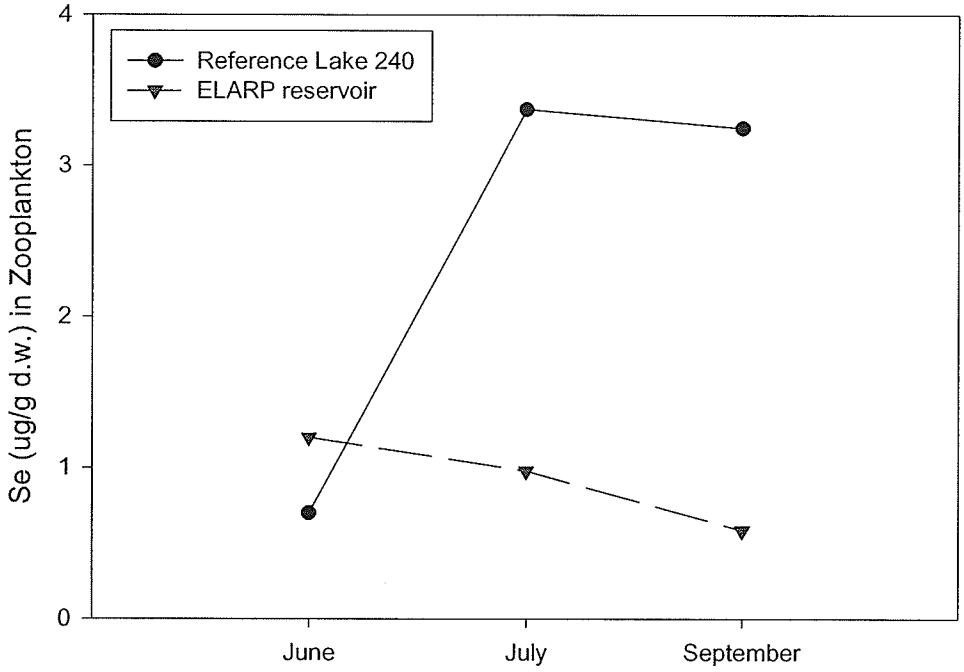


Figure 3. Concentrations of Se in zooplankton in June, July, and September in the ELARP and FLUDEX reservoirs. Each data point is the concentration in one sample.



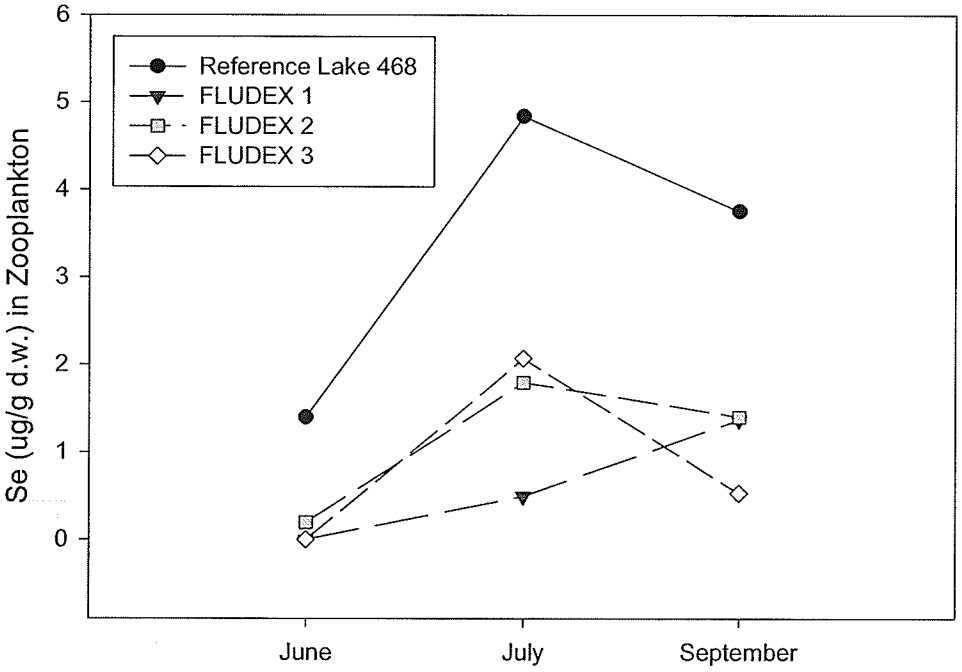
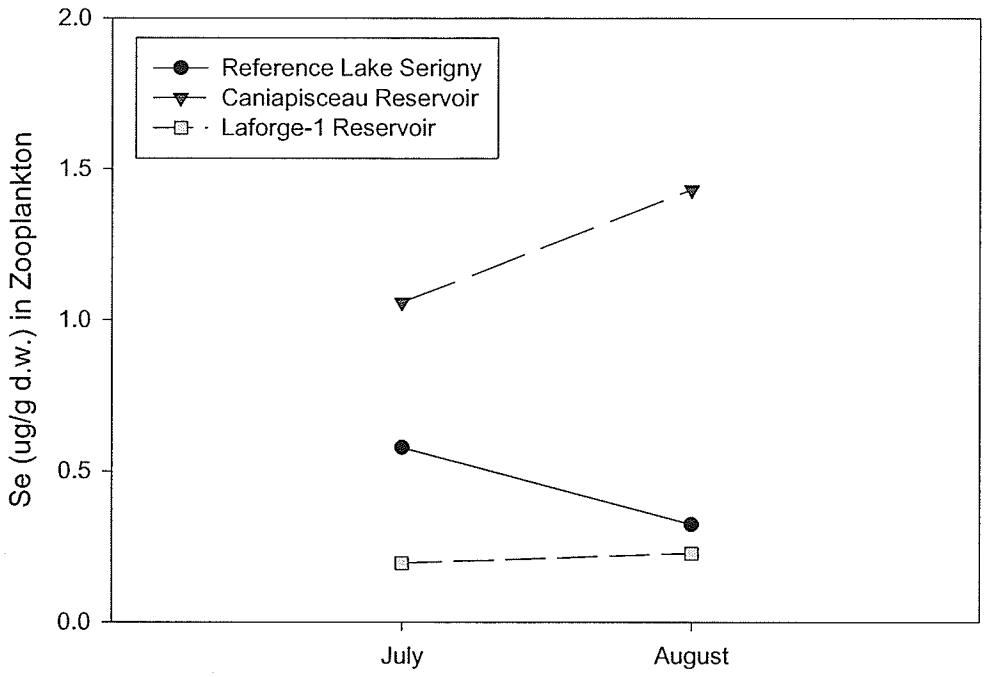


Figure 4. Concentrations of Se in zooplankton from La Grande Complex and Robertson Reservoir in July and August. Each data point is the concentration in one sample.



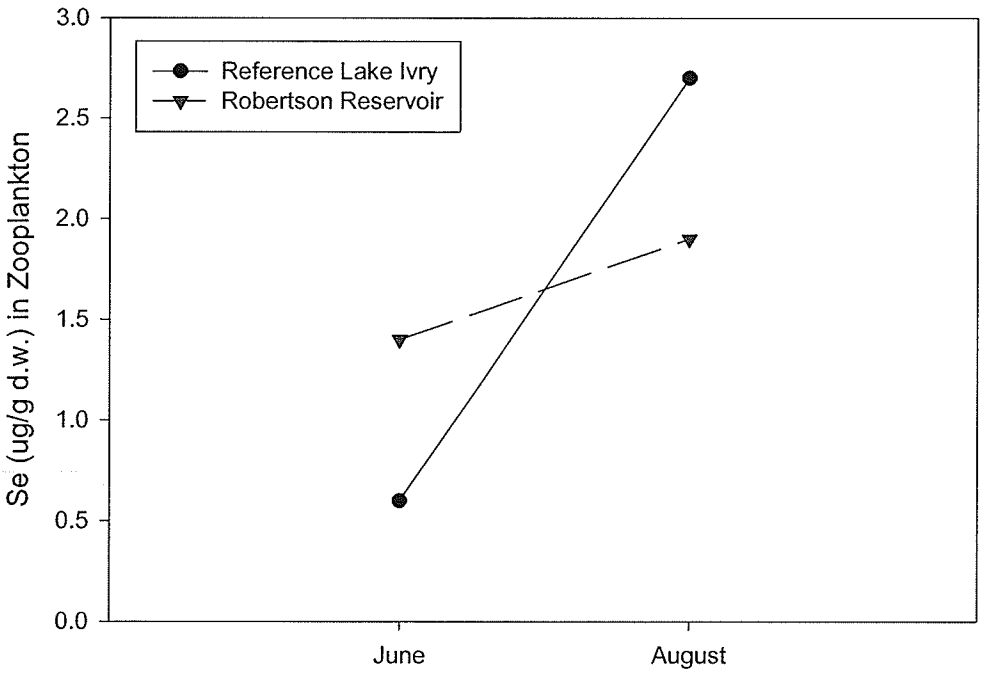
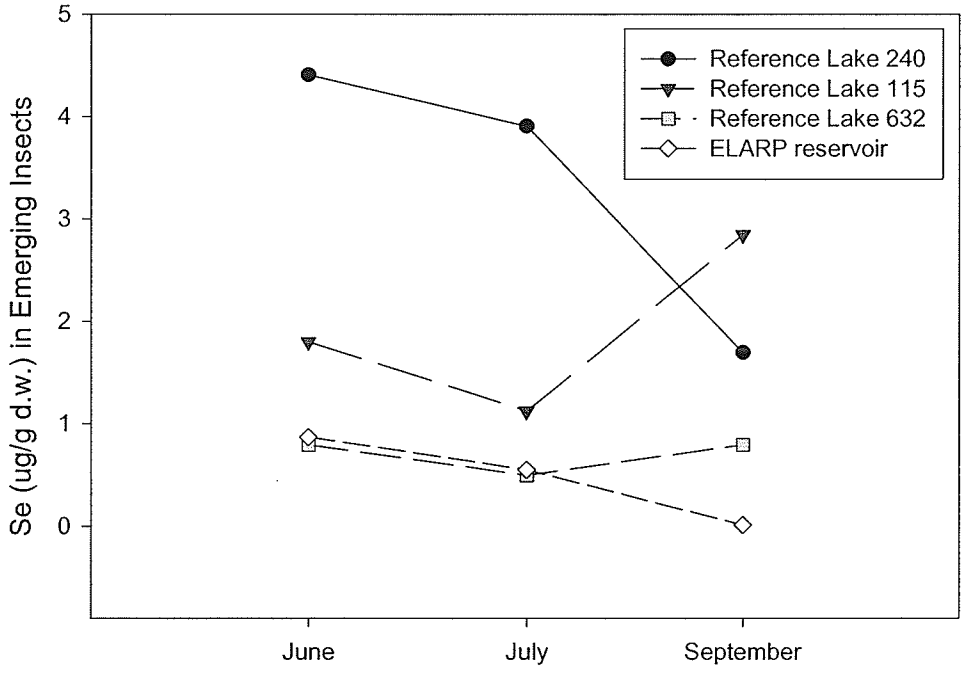


Figure 5. Concentrations of Se in emerging insects from the ELARP and FLUDEX reservoirs in June, July, and September. Each data point is the concentration in one sample. There was insufficient sample mass to analyze selenium in Reference Lake 468. Emerging insects from Reference Lakes 115 and 632 were used for comparison.



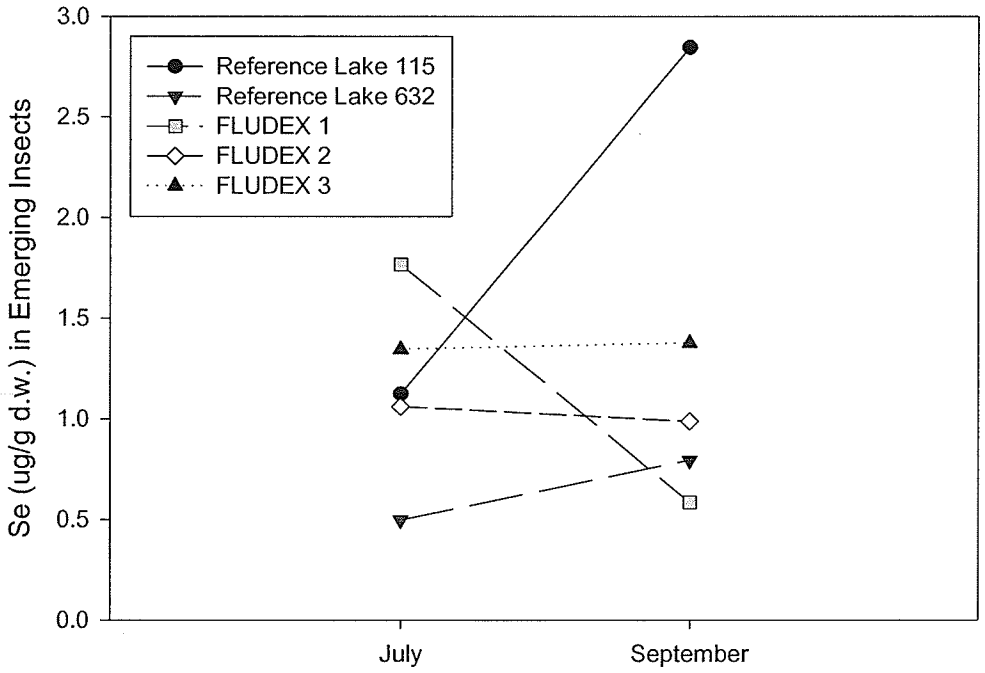


Figure 6. Concentrations of Se in Chironomid larvae from Québec in July and August. Each bar is the concentration of one sample.

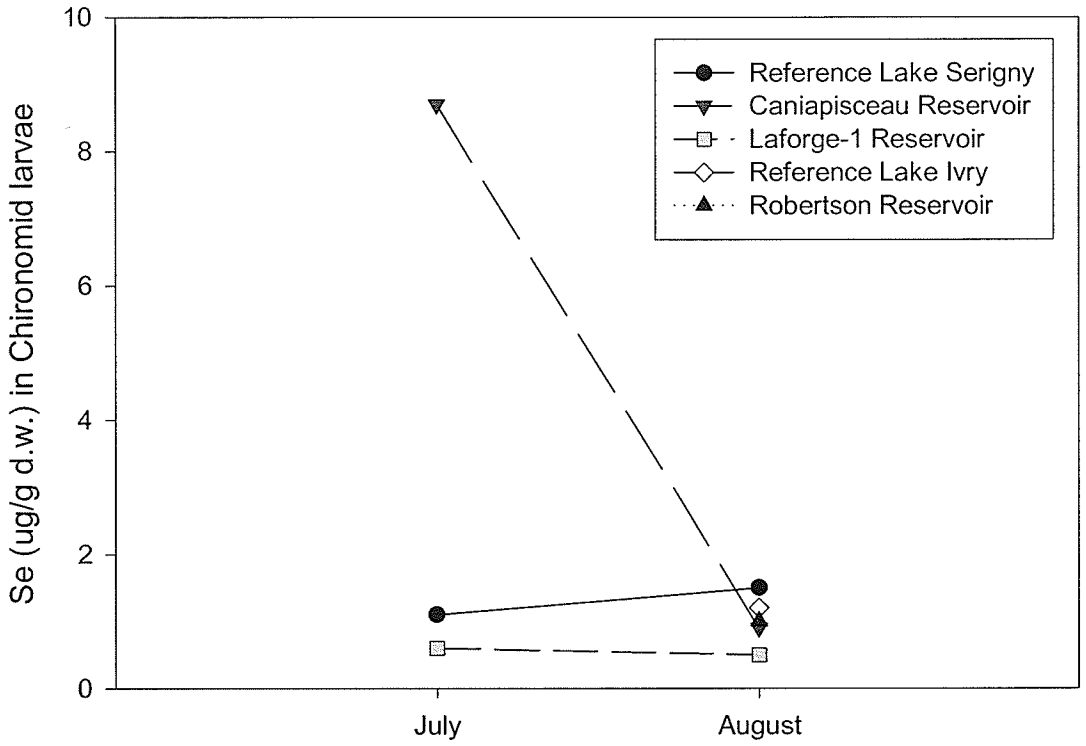
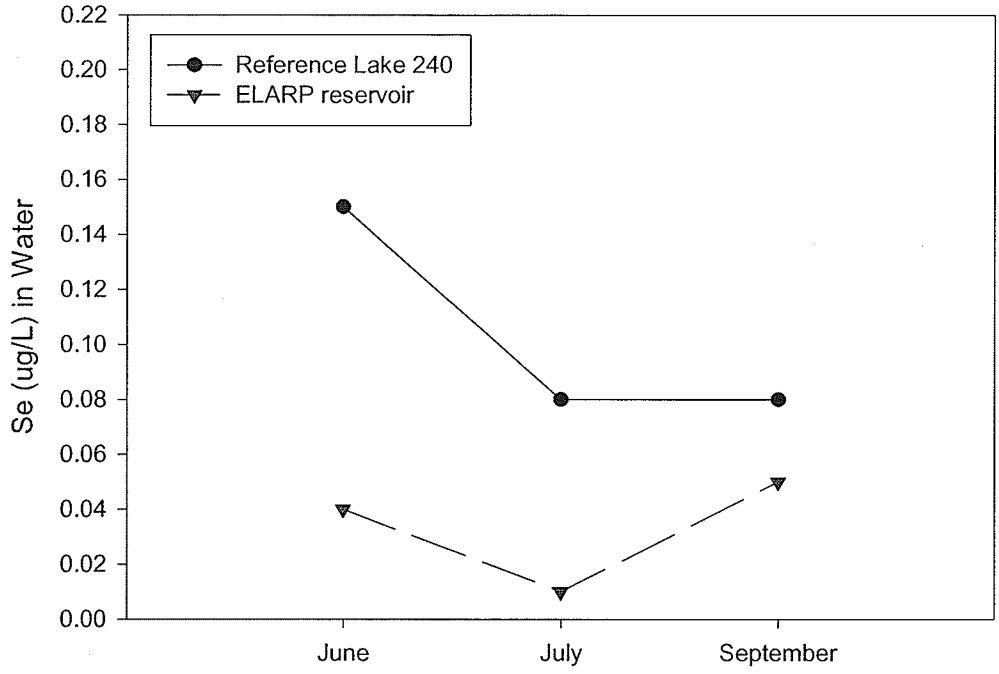


Figure 7. Concentrations of Se in surface water from the ELARP and FLUDEX reservoirs. Each data point is the concentration in one sample.



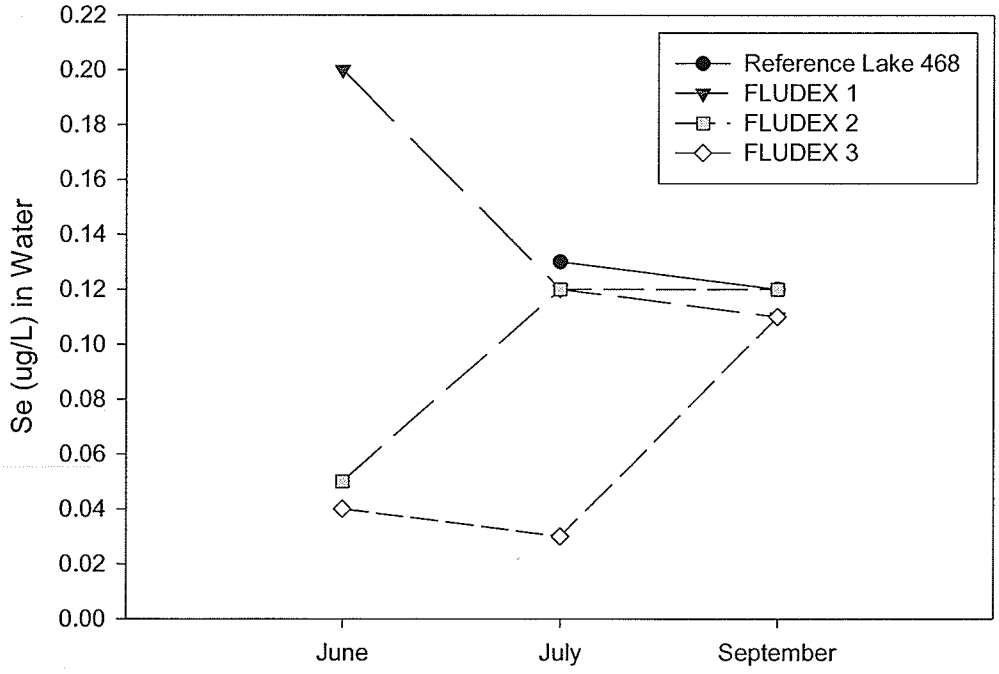


Figure 8. Concentrations of Se in surface water from Québec. Each data point is the concentration in one sample.

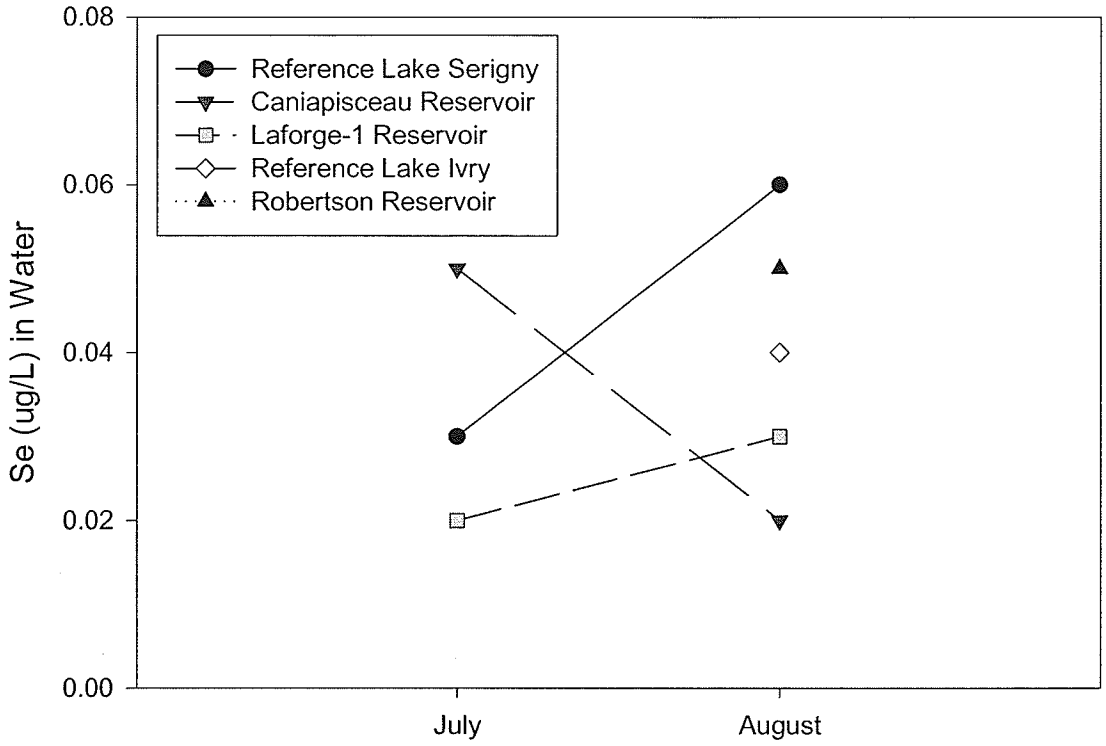


Figure 9. Concentrations of Se in soil from Ontario and Québec. Each bar is the mean concentration in three samples. Error bars are one standard deviation.

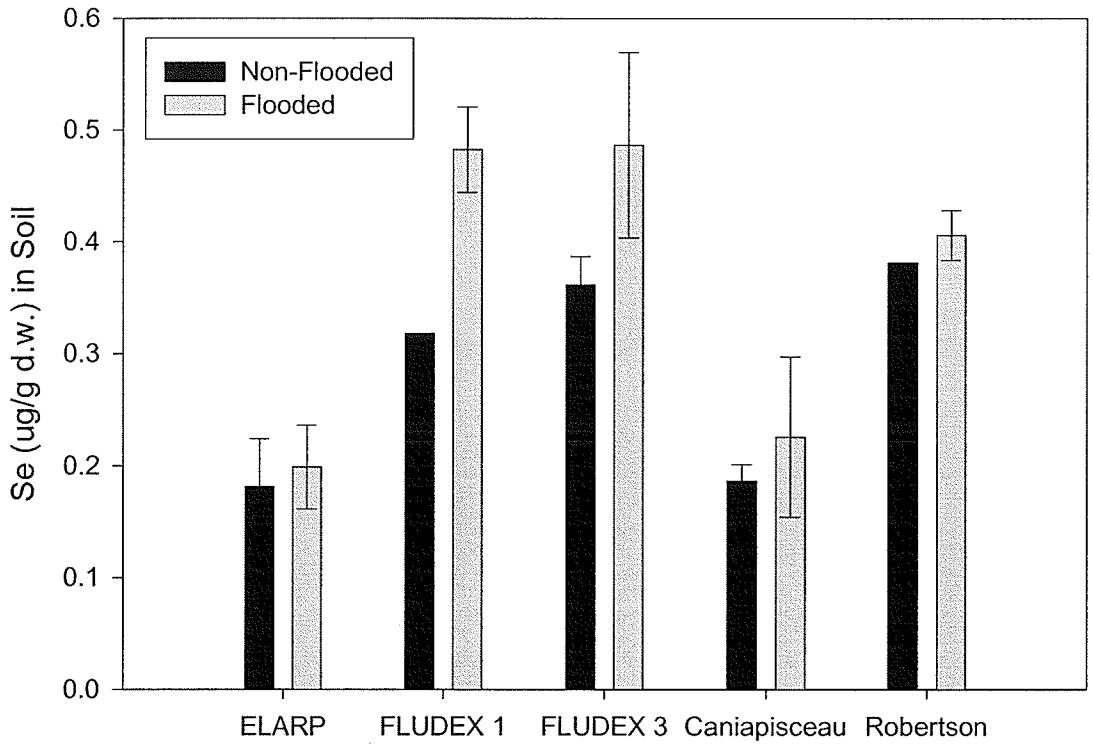


Figure 10. Concentrations of Hg versus concentrations of Se in muscle of finescale dace from the ELA in September. Each data point represents one sample.

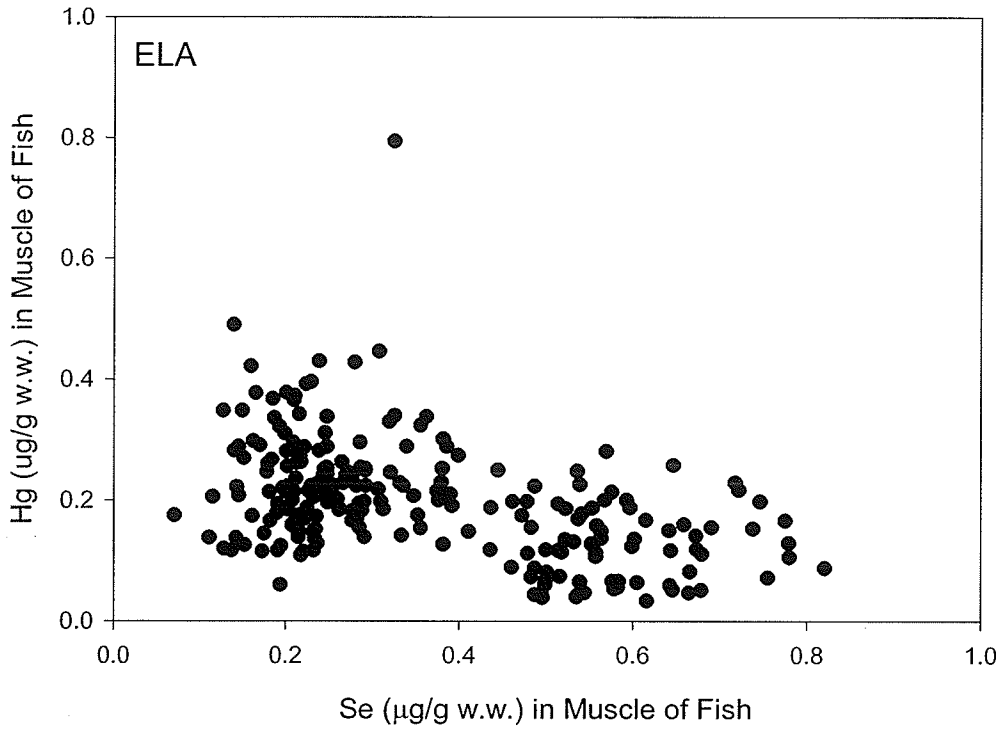
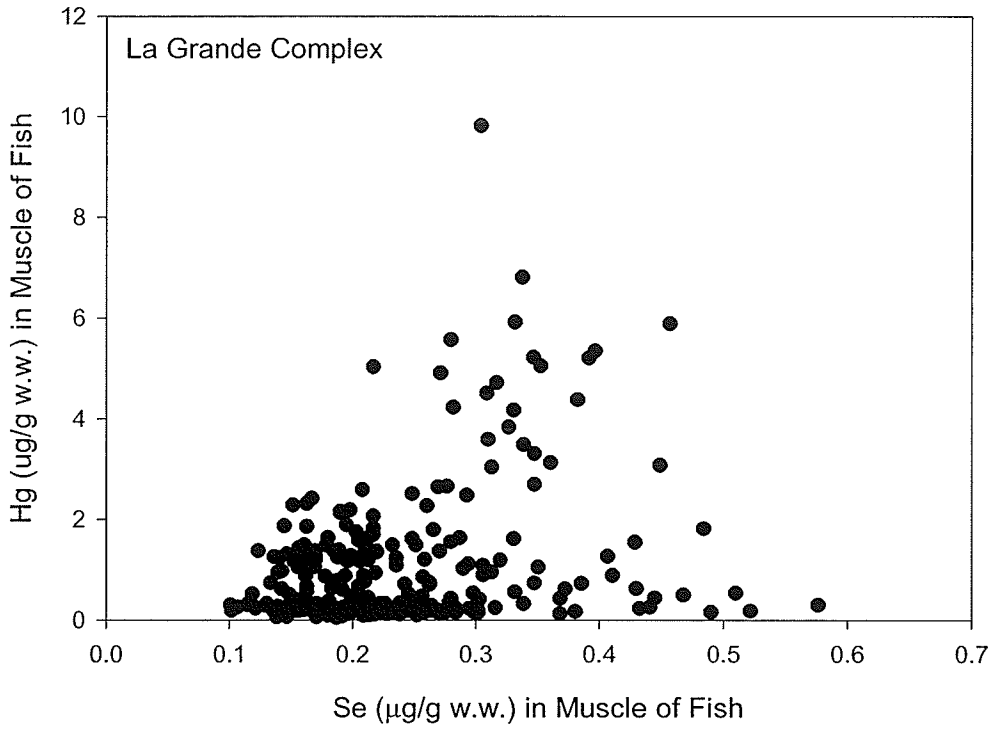


Figure 11. Concentrations of Hg versus concentrations of Se in muscle of fish from Québec. Each data point represents one sample.



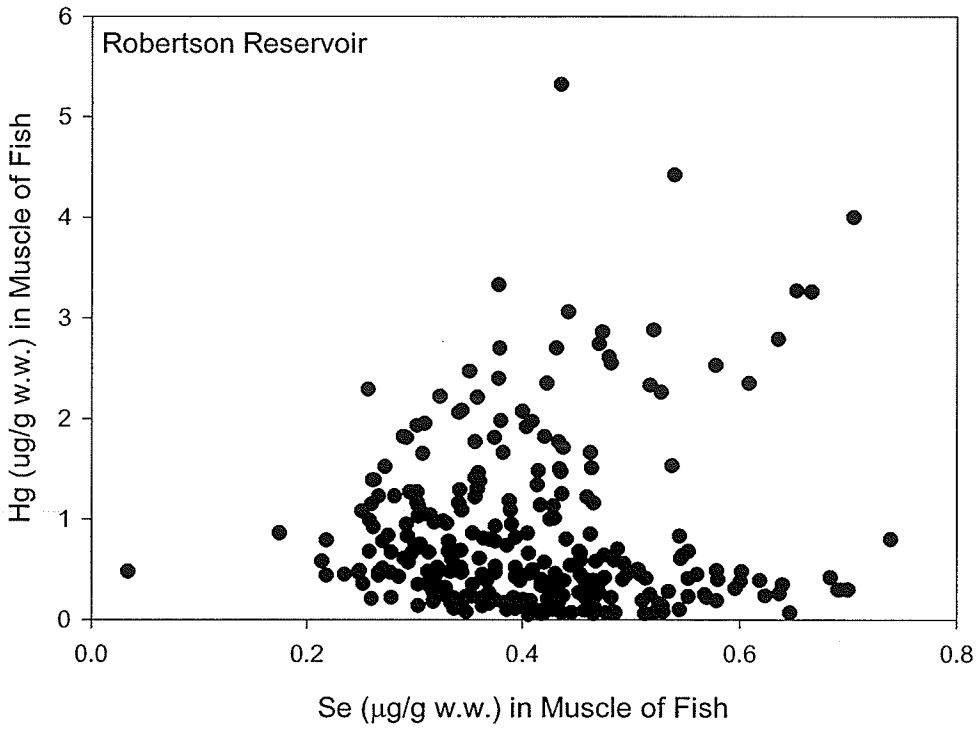


Figure 12. Concentrations of MeHg versus concentrations of Se in zooplankton from the ELA in September and in Québec in August. Each data point represents one sample.

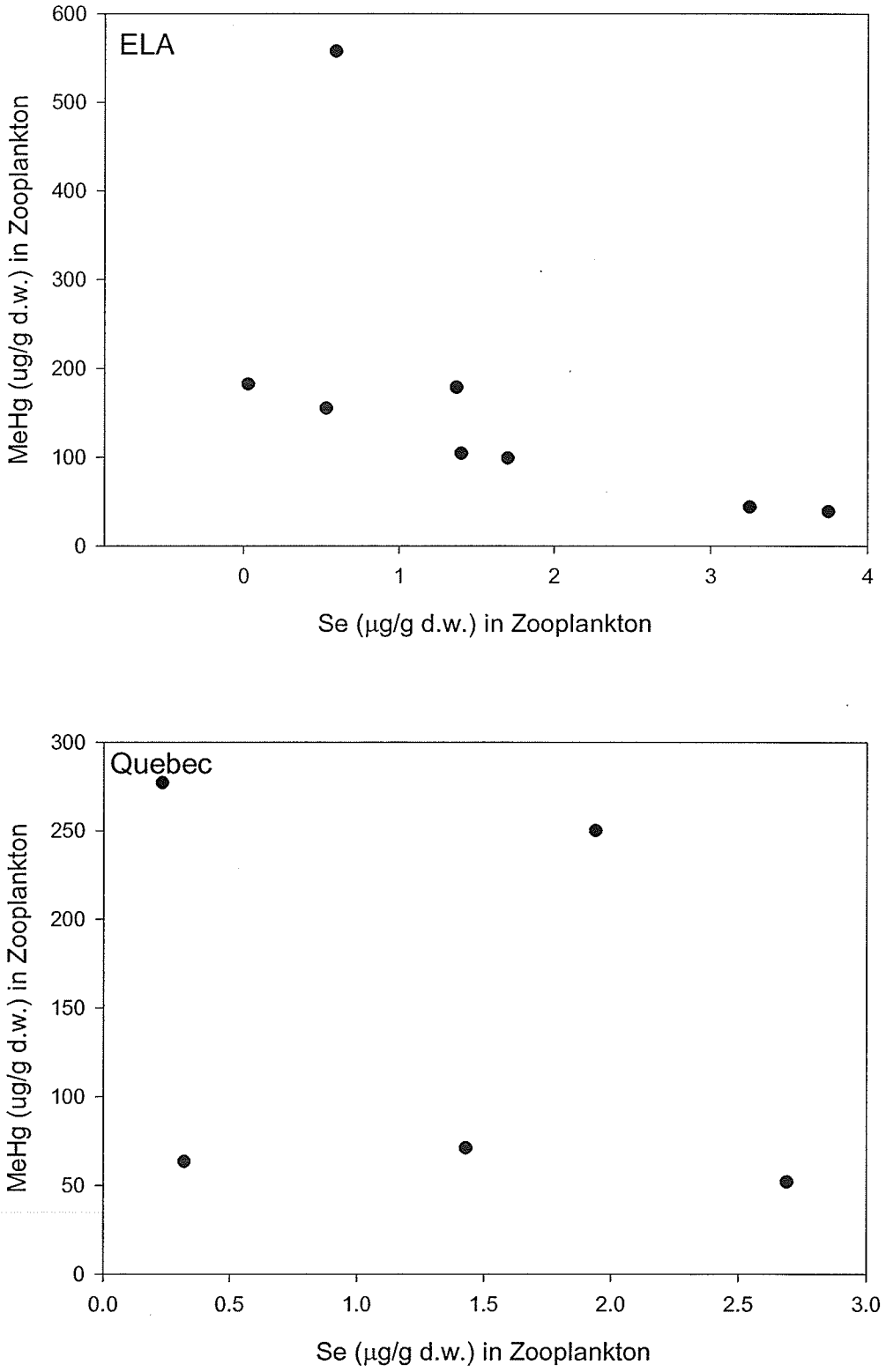


Figure 13. Concentrations of MeHg versus concentrations of Se in emerging insects from the ELA in September. Concentrations of MeHg versus concentrations of Se in Chironomid larvae from Québec in August. Each data point represents one sample.

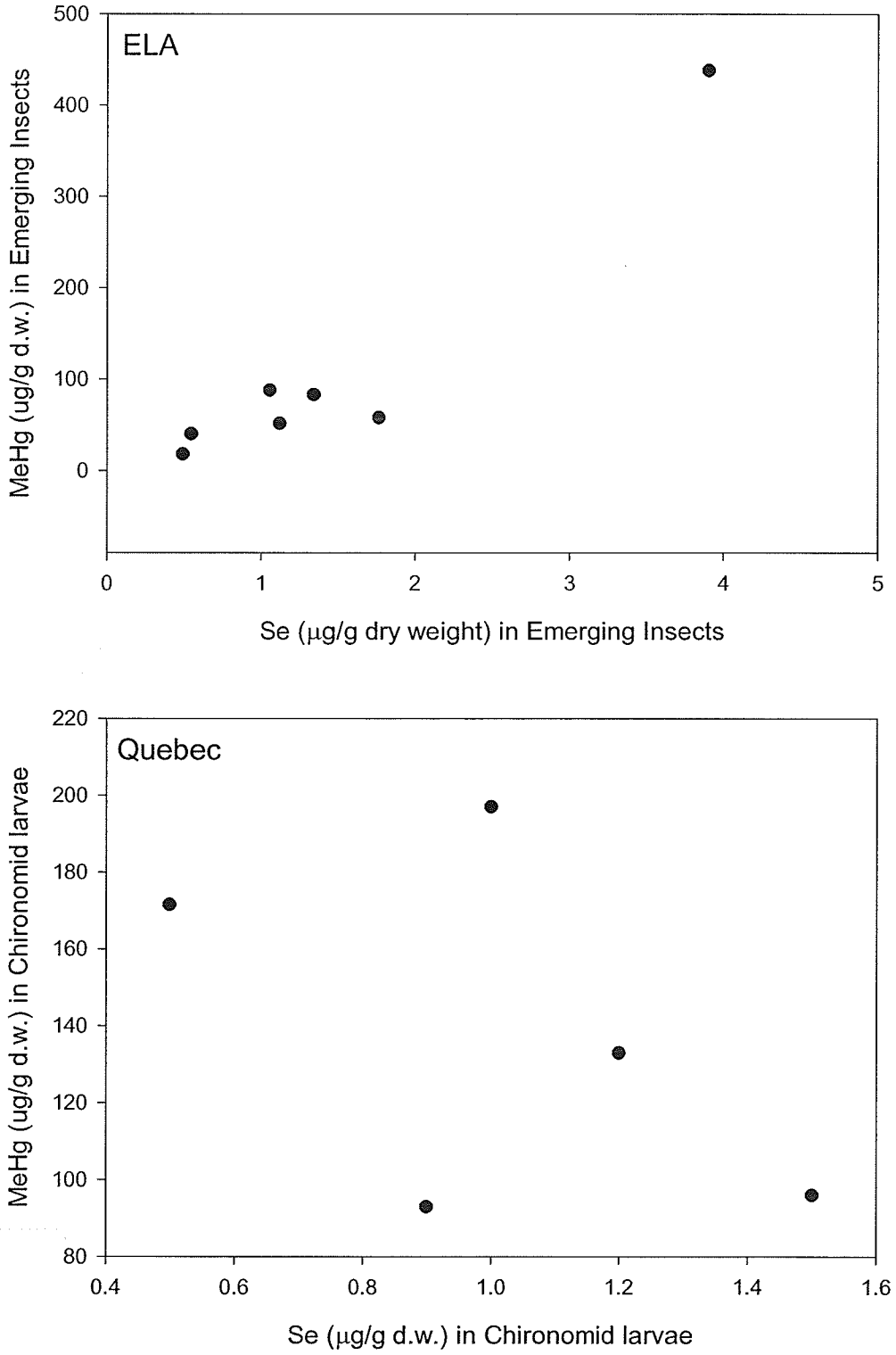
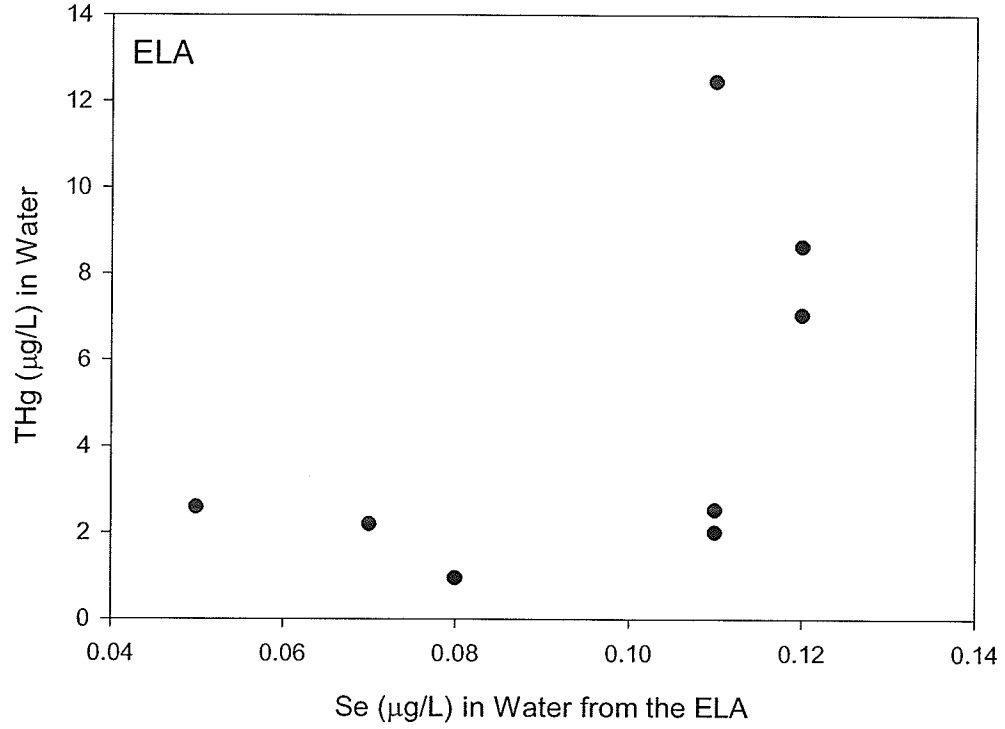


Figure 14. Concentrations of Hg and MeHg versus concentrations of Se in water from the ELA in September. Each data point represents one sample.



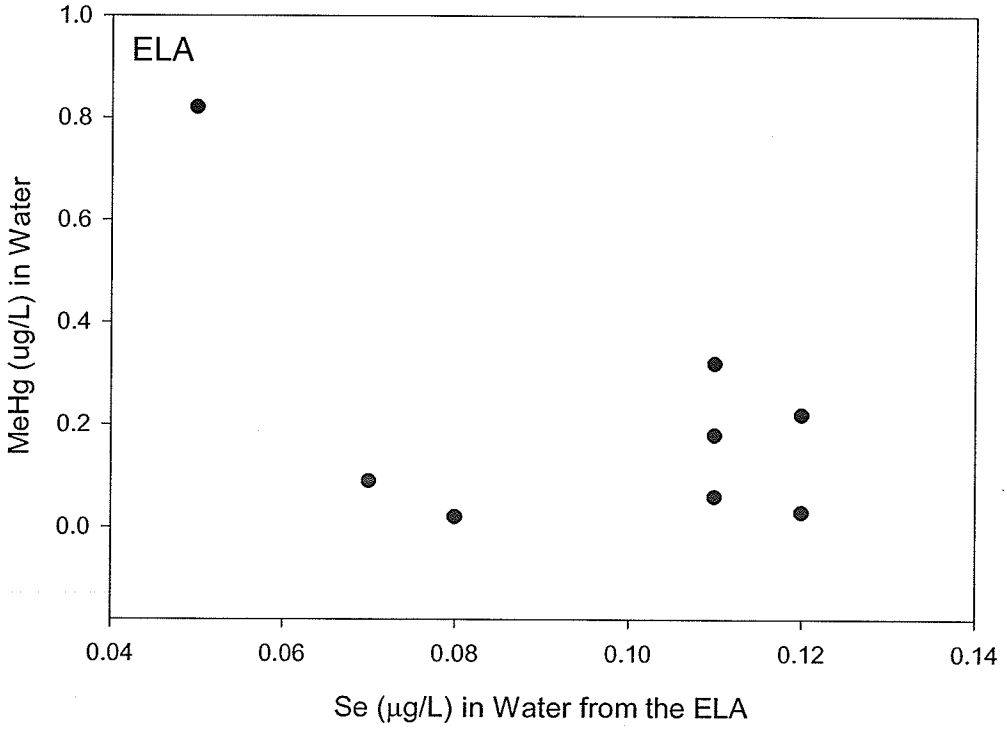


Figure 15. Concentrations of Hg and MeHg versus concentrations of Se in water from reservoirs in Québec in August. Each data point represents one sample.

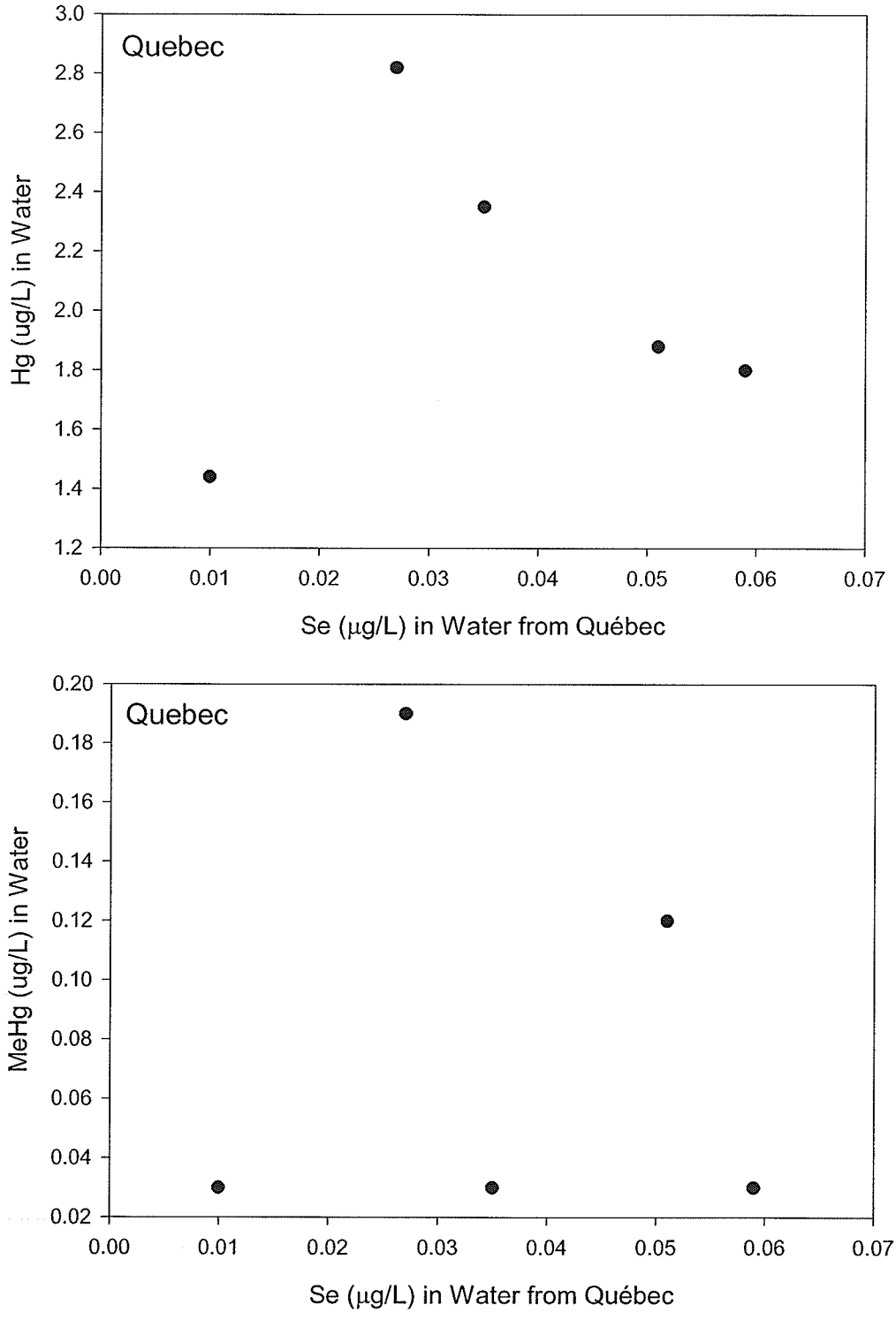
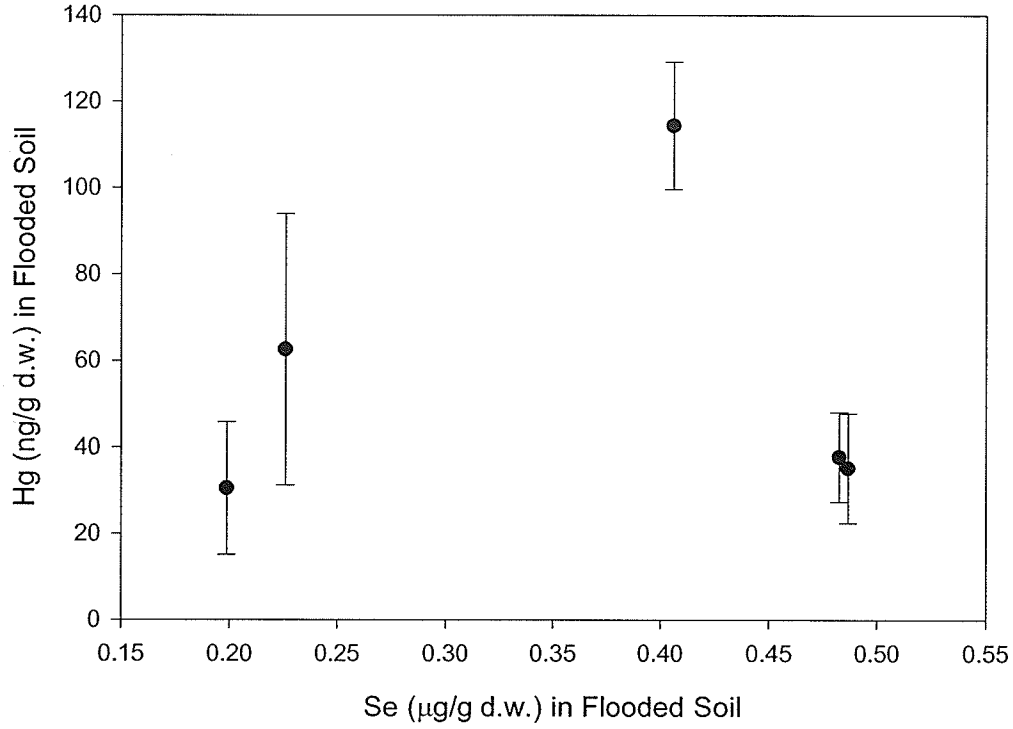
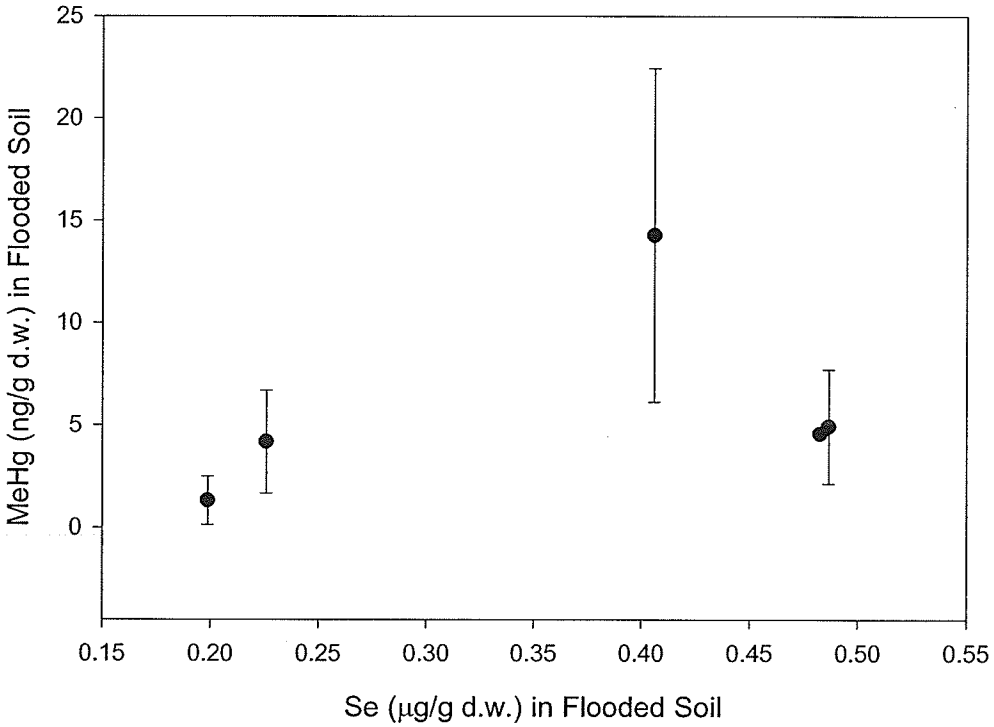


Figure 16. Concentrations of Hg and MeHg versus concentrations of Se in flooded soil in Caniapisceau, Robertson, FLUDEX 2, FLUDEX 3, and ELARP reservoirs. Each data point represents the mean concentration of three samples with one standard deviation.





FATES AND MASS BALANCES OF SELENIUM AND ISOTOPIC MERCURY ADDED TO MESOCOSMS

ABSTRACT

Our overall goal was to understand the effectiveness of adding selenium (Se) at environmentally relevant concentrations to lake water on mercury (Hg) concentrations in fish. The specific objective of this paper was to determine the fate of Hg and methyl mercury (MeHg) in filtered water, particulate matter, and surface sediment, the fate of added Se, and to evaluate whether added Se affected the partitioning of Hg among these compartments. Three forms of Se, total Se, Se^{4+}, and Se^{6+}, were analyzed to determine if a specific form of Se affected Hg cycling. We added different doses of Se to six large mesocosms in a freshwater lake, sampled filtered water, particulate matter, and surface sediment, and then analyzed the data using regressions, and constructed a mass balance for Hg. We added the same amount of labelled ^{198}Hg to each mesocosm. Actual Se concentrations in filtered water were positively related to the intended Se concentrations in July ($p=0.006$).

The fate of Se added to freshwater mesocosms was challenging to interpret because it was indistinguishable from ambient Se. In July, concentrations of dissolved Se^{6+} ($p=0.008$) and in August concentrations of TSe ($p=0.006$) and Se^{4+} ($p=0.004$) associated with particulate matter were significantly related to concentrations of TSe in filtered water. The partitioning of Se to particulate matter seemed to depend on the amount of Se added to these systems. More TSe and Se^{4+} partitioned to particulate matter with increasing Se concentrations in water while the opposite occurred with Se^{6+}. A lack of relationship between Se species in surface sediment and TSe concentrations in filtered water may be explained by the relatively short duration of the experiment. Presumably, there were large amounts of Se in the surface sediment at the beginning of the experiment and the added Se was small in relation to this mass. The greatest mass of Se was found in surface sediment and the second greatest mass was dissolved in water. The Hg results were as follows: in August, concentrations of ambient MeHg in filtered water were positively related to Se concentrations in filtered water ($p=0.004$), indicating that more MeHg was in aqueous form at higher concentrations of Se. This may indicate that small amounts of Se added to lake water stimulated MeHg production or distribution. In addition, concentrations of ambient THg on particule matter ($p=0.02$) were positively related to Se concentrations in filtered water and this suggests that total Hg was more mobile or available for uptake at higher concentrations of Se in water. Selenium did not affect ambient or spike THg

and MeHg concentrations in any other compartments. The greatest sink for spike THg was the unknown portion which we assume to be evasion to the atmosphere. In order of importance, the remaining sinks for spike THg were surface sediment, dissolved in water, and associated with suspended particulate matter. The distribution of Hg among abiotic compartments at the end of our study was not related to Se treatments and therefore we conclude that Se did not affect the partitioning of Hg. The potential for modifying Hg bioaccumulation was not determined by abiotic Se dynamics.

INTRODUCTION

Exposure to mercury (Hg) as methyl mercury (MeHg) from eating fish can cause toxicity to people and wildlife. Fish acquire most of their MeHg burden through their diet (Hall *et al.* 1997; Bodaly and Fudge 1999) while the lower food web may absorb MeHg directly from water (Monson and Brezonik 1998; Peech Cherewyk 2002) and from diet (Mason *et al.* 1996; Plourde *et al.* 1997; Hall *et al.* 1998). The level to which MeHg bioaccumulates depends partly on the conversion of inorganic Hg to MeHg by microorganisms at the oxic-anoxic interface in lake sediments or hypolimnetic water (Compeau and Bartha 1985; Pak and Bartha 1998). Also, MeHg can undergo photo- and biological demethylation to ionic or elemental Hg (Sellers *et al.* 1996; Eckley *et al.* 2005). Reactive gaseous Hg can then evade to the atmosphere (Sellers *et al.* 1996; Amyot *et al.* 2004; Eckley *et al.* 2005), while Hg associated with particulate matter may settle to the lake bottom (Hurley *et al.* 1991; Hesslein *et al.* 1980).

Modification of the Hg cycle by adding Se may lower Hg concentrations in fish by slowing Hg uptake by fish or the lower food web (Rudd *et al.* 1980; Turner and Swick 1983; Turner and Rudd 1983), facilitating Hg loss from fish (Pedersen *et al.* 1998; Bjerregaard *et al.* 1999), or depressing Hg methylation by forming insoluble Hg compounds (Jackson 1991; Chen *et al.* 1997; Jin *et al.* 1999). However, the relative importance of these mechanisms is not well understood. In addition, the most relevant form of Se that affects Hg cycling has not been determined. The benefits of adding Se to lower Hg concentrations are the quick response of the system, ease of application, and low cost. However, the hazards of Se additions are reproductive toxicity to fish and wildlife in the immediate site of application or downstream (Lemly 1996; Lemly 1997; Lemly 2002a) and the precedent for other deleterious substances to be added to ecosystems.

Selenium occurs naturally, although locally elevated concentrations exist due to human activities (reviewed by Maier and Knight 1994 and Presser *et al.* 1994). Inorganic selenate (Se^{6+}) and selenite (Se^{4+}) are soluble and can be converted to selenide by methylating microorganisms in anaerobic lake sediment (Chau *et al.* 1976) and other organic forms (selenocysteine and selenomethionine) after assimilation from water by primary producers (Besser *et al.* 1993, Baines and Fisher 2001). In addition, primary producers also assimilate dissolved organic Se species (Baines *et al.* 2001). Formation of organic forms of Se is essential to Se bioaccumulation and therefore we analyzed selenate and selenite to determine how available the Se is for uptake. Methylated selenide is insoluble and thus evades to the atmosphere where it is photo-oxidized (Chasteen and Bentley 2003). Selenoamino acids form

proteins and bioaccumulate (Hodson and Hilton 1983; Bertram and Brooks 1986; Pedersen *et al.* 1998; Peters *et al.* 1999). Therefore the form of Se may be important for determining the efficacy of Se bioaccumulation and dynamics between Se and Hg cycles.

Our objectives were to determine the fate of Hg and Se added to freshwater and whether added Se affected the THg or MeHg distribution. Our null hypotheses were 1) Hg and Se concentrations and partitioning among abiotic compartments were not related to Se concentrations in filtered water and 2) MeHg concentrations in environmental compartments were not related to Se concentrations in filtered water. We used large mesocosms in a natural, freshwater lake to simulate lake environments to which we added isotopic Hg and a range of low concentrations of Se. We monitored the concentrations of these chemicals for eight weeks in surface sediment, filtered water, and particulate matter. We report concentrations and amounts of Hg and Se in each compartment, describe relationships between concentrations and Se dose, and discuss Hg and Se partitioning and mass balances.

METHODS

Location and Materials

This experiment was conducted during the summer of 2005 in an oligiotrophic lake, Lake 239, at the Experimental Lakes Area (ELA; 49°40' N, 93°44' W) that was surrounded by jack pine and white birch forest (Brunskill and Schindler 1971). Six mesocosms 10 m in diameter and with a maximum depth of 2.8 m were deployed in a sheltered bay with sandy sediment (0.2 to 1.3% carbon) near the main inflow to the lake. They consisted of plastic walls sealed to sediment with sandbags and attached to floating collars. We rinsed mesocosm walls in lake water before deploying. The approximate volume of the mesocosms was 196 350 L, calculated using the mean depth. We assumed leakage was less than 10% (Orihel 2005).

In filtered water, background concentrations of total mercury (THg) were 1.7 to 2.5 ng/L. In particulate matter, initial concentrations of THg ranged from 82 to 112 ng/g. Concentrations of MeHg in filtered water before treatment addition were 0.1 to 0.7 ng/L. In June, THg that occurred as MeHg ranged from 0.5 to 2.6% in filtered water. Concentrations of THg and MeHg in filtered water had no relationships with the position of the mesocosms.

Addition of Hg and Se

Ambient Hg is defined as the Hg naturally present in the systems before treatment addition whereas spike Hg is the isotopically lablled portion of Hg that we added. We added a

stable isotope of Hg (93.53% ^{198}Hg) as mercuric chloride (HgCl). We will refer to the stable isotope of ^{198}Hg as spike Hg. Spike Hg was added as a tracer that could be analyzed separately from ambient Hg. Therefore, spike Hg was used to monitor the effects of adding Se. Even though only a small amount of the spike Hg was methylated and returned to the water, this small amount of spike methyl mercury (MeHg) was an important source of MeHg to the food web. We added 5.8 mg (0.3 μg/L) of spike Hg to each mesocosm. This amount was about ten times the mean annual atmospheric deposition of 7 μg/m^2·y at the ELA (St. Louis *et al.* 2001). The isotopic Hg was dissolved in nitric acid (5%) and then measured into Teflon bottles. This solution was mixed with about 500 mL of water from Lake 239 the night before treatment addition.

We used a regression-based model for our study design. We added 0, 0.1, 0.2, 0.4, 0.8, and 1.6 μg/L of sodium selenite (Na_2SeO_3) to different mesocosms. Concentrations of Se were known to be low in surrounding lakes, but they varied seasonally (Mailman and Bodaly, unpublished data) and we assumed Se concentrations in water of approximately 0.1 μg/L. Treatments were randomly assigned to mesocosms at concentrations ranging from 1 to 16 times natural background concentrations. We added treatments on 22 June 2005 after sunset to avoid photodegradation. We released the treatments into the prop-wash of an electric trolling motor. We added spike Hg to all mesocosms, waited an hour, and then added Se to all mesocosms.

Sample Collection

Filtered Water and Particulate Matter

Water samples were collected on 21 June (pre-treatment), 19 July, and 16 August for analyses of THg, MeHg, TSe, Se^{4+} and Se^{6+}. Using a battery-powered peristaltic pump, water samples were pumped from 1 m depth at the centre of each mesocosm thorough Teflon tubing and a pre-cleaned quartz fibre filter (QM-A; 0.7 μm nominal pore size) into pre-washed glass containers (40 mL for THg, 250 mL for MeHg, and 500 mL for Se). Clean techniques were used (St. Louis *et al.* 1994). Water samples were acidified with 160 μL of concentrated hydrochloric acid (HCl; Baker), 1 mL of concentrated HCl (Baker), and 2.5 mL of 37% trace metal grade HCl (Fisherbrand), respectively. Filters were stored frozen at -4°C for less than two years. Concentrations of Hg and Se in particulate matter and surface sediment were standardized to carbon concentrations. Carbon was analyzed by standard methods at the ELA chemistry laboratory (modified from Hauser 2001 and Stockner and Armstrong 1971).

Samples of water for chemical analyses were collected concurrently with other water samples in acid-washed high-density polyethylene bottles. The ELA chemistry laboratory analyzed these samples for suspended and dissolved ions, major nutrients, and acidity according to Stainton *et al.* (1977).

Surface Sediment

On 12 July, surface sediment was collected for pre-treatment analyses of THg, MeHg, and Se from outside of mesocosms to avoid disturbing the experiment. Final samples of surface sediment were collected last to avoid disruption of other compartments. Three sediment samples from each mesocosm were collected using 7 cm diameter high density polyethylene (HDPE) pipe that was cut into 20 cm lengths and beveled on one end. Wearing SCUBA gear, we pressed these pipes into the sediment at random locations within the mesocosms. Then we inserted a rubber stopper at the top, lifted the tube, and inserted another stopper in the bottom. Sediment samples were transported to the laboratory in a cold, dark cooler. Water was decanted, and then the top two cm of the sediment was ejected using a silicone stopper mounted on PVC pipe. If plants were present, they were not removed. Sediment samples were frozen at -4°C for less than one year in polyethylene bags. They were weighed before and after freeze drying to calculate wet to dry weight ratios.

Analyses

Analyses of Hg

Mercury was detected by inductively coupled plasma mass spectrometry (ICP-MS) at Trent University in Peterborough, Ontario. Each sample was spiked with 100 µL of ~60 ng/mL of internal Hg standard (2.3 ng/mL of $^{201}Hg^{2+}$). Half of the quartz-fibre filters containing particulate matter were spiked with 20 µL of internal Hg standard. Samples of particulate matter on filters were digested with 1 mL of 7:3 HNO_3:H_2SO_4 for two hours at 120°C on an aluminium block. Samples of surface sediment were digested with 7:3 HNO_3:H_2SO_4 at 80°C. Filters were preserved with 0.5 mL of BrCl and diluted with 10 mL of Milli-Q water. Mercury in samples of filtered water (20 mL) was oxidized to Hg^{2+} using 100 µL of 0.2 N BrCl and 20 µL of the standard 0.48 ng/mL $^{201}Hg^{2+}$ under a laminar flow hood for 12 hours.

Samples of filtered water and particulate matter were analyzed for THg in the same manner. Mercury-II in digestate was reduced to Hg^0 using 3% (w/v) $SnCl^2$. Mercury-II in samples of surface sediment was reduced with sodium borohydride (details are provided by

Hintelmann *et al.* 2002). Gaseous elemental Hg^0 was separated from solution by a cold-vapour generation system, and then gaseous Hg^0 was purged with Hg-free argon gas and detected on an ICP-MS (Hintelmann and Ogrinc 2003). This datum included all Hg isotopes in each sample. The concentration of spike Hg was calculated by multiplying the measured concentration of ^{198}Hg isotope by the percent of ^{198}Hg that was present in Hg added to mesocosms.

Distillation of MeHg occurred by transfer of Hg vapour in sample test tubes to receiving test tubes to separate MeHg from its matrix. In all cases, 5 mL of Milli-Q water was added to receiving test tubes. To 50 mL of samples of filtered water there was added 20 µL of 1 ng/mL of $^{201}MeHg$ solution, 500 µL of 9M H_2SO_4, and 200 µL of 20% KCl. Samples of filtered water were distilled at 140°C for 5 to 7 hours. For samples of particulate matter and surface sediment, half the filter and 200 mg of sample, respectively, were placed in individual test tubes to which were added 20 µL of 1 ng/mL $^{201}Hg^{2+}$, 10 mL of Milli-Q water, 0.5 mL of H_2SO_4, and 0.2 mL of KCl. These samples were distilled at 140°C for 1 hour and 30 minutes. 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 (Hintelmann and Evans 1997; Horvat *et al.* 1993).

No sample was added to digestion blanks. Digestion and filter blanks were also analyzed. The limit of detection (LOD) was 0.2 and 0.1 ng/L for ambient THg in filtered water and particulate matter and 0.15 ng/g in surface sediments. For MeHg the LOD was 0.02 and 0.002 ng/L in filtered water and particulate matter and 0.04 ng/g in surface sediments. Spike THg and MeHg LODs were 0.5% of the LODs for ambient THg and MeHg. Certified reference material (CRM) were prepared and digested in the same manner as samples. MESS-3 was the CRM for THg in particulate matter and surface sediment and its measured values were not significantly different from the expected values.

Analyses of Se

Selenium was detected by atomic fluorescence hydride generation spectroscopy using a PS Analytical Millennium Excalibur Model 10.005 at Flett Research, Ltd., Winnipeg, Manitoba. For each sample of water, TSe, Se^{4+}, and Se^{4+} plus Se^{6+} were analyzed. To measure Se species, 9.35 mL of each sample of filtered water was heated to 93 to 97°C for 15 minutes, and then cooled. Samples were adjusted to 4 N HCl at room temperature and analyzed to determine Se^{4+}. To determine Se^{4+} plus Se^{6+}, the sample was adjusted to 4 N HCl, reheated to 94 to 97°C for 15 minutes, cooled, and then analyzed. The concentration of Se^{6+} was calculated by subtracting

Se^{4+} from Se^{4+} plus Se^{6+}. To measure TSe, 200 µL of BrCl was added to 9.35 mL of each sample of filtered water. The solution was heated at 93 to 97°C for 15 minutes and then cooled. It was adjusted to 4 N HCl, reheated in the same manner, cooled, and then analyzed.

Samples of particulate matter (0.05 g) were digested with 5 mL of nitric-sulphuric acid for 1 h at room temperature. They were then heated to 150°C for six hours, cooled, and then diluted to 10 mL with deionized water. Then 1 mL of digest solution plus 8.35 mL of deionized water was heated to 93 to 97°C for 15 minutes, cooled, adjusted to 4 N HCl, and Se^{4+} was analyzed. For Se^{4+} plus Se^{6+}, the above process was followed by reheating in the same manner, cooling, and then analyzing. 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 to 97°C for 15 minutes, cooled, adjusted to 4 N HCl, reheated in the same manner, cooled, and analyzed.

Each analytical run included two matrix spikes and their duplicates, a duplicate of one sample, and duplicates of CRM. Analytical blanks were treated the same as the tissue samples. Measurements of Se in samples were repeatable. Triplicate analysis of water had a mean recovery of 106.3%. The CRM for surface sediment was MESS-2. Its certified value is 0.72 +/- 0.09 µg Se/g (standard deviation). We measured 0.69 +/- 0.01 µg/g (95.8% recovery). The CRM for particulate matter was DORM-2. Its certified value is 1.4 +/- 0.09 µg/g. We measured 1.36 +/- 0.04 µg/g (97.1% recovery). For filtered water the CRM is TM-28.2 lot 603 (Environment Canada National Water Research Institute in Burlington, Ontario) with a certified value of 3.6 µg/L +/- 0.96 µg/L. We measured 3.24 +/- 0.12 µg/L (90% recovery) and 3.76 +/- 0.37 µg/L (104.4 % recovery). Measured values of the CRMs were not significantly different from the expected values.

Data Analyses

Simple linear regressions modeled the relationship between THg or MeHg concentrations in environmental compartments and TSe concentrations in water. Mean total Se concentrations in filtered water from 19 July and 16 August were used as the independent variable to most accurately approximate Se concentrations. All data were $\log_{10}$ transformed before running statistical analysis using Statistical Analysis Software (SAS) version 9.1.

We calculated mass balance budgets for spike THg in all mesocosms eight weeks after treatment addition. Masses of spike THg in filtered water, particulate matter, and surface sediment were included in our budget. We did not include concentrations of Hg found in

periphyton on mesocosm walls, but it was found to be small in other studies (Orihel et al. 2006). The masses of spike THg in filtered water and particulate matter were calculated by multiplying the concentrations of spike THg by the volume of water in the given mesocosm. Volume was estimated by adding NaCl after the final sampling was complete. The concentrations of NaCl were divided into the mass of salt added to estimate the volume of water in which the NaCl was diluted. The volume estimates ranged from 209,639 +/-9047 to 238,317 +/- 1702 L. The mass of spike THg in sediment was calculated by multiplying the concentration of spike THg by the mass of surface sediment to 2 cm depth. The mass balance was determined by adding the masses of spike THg in filtered water, particulate matter, and surface sediment and then subtracting the mass of spike THg added to the mesocosms. We assumed the missing portion evaded to the atmosphere. Biota were not included because they accounted for a minute percentage of Hg in previous mass balance studies (Hesslein *et al.* 1980; Hall *et al.* 2005; Orihel *et al.* 2006).

RESULTS

For each environmental compartment, we report whether spike Hg was detected, relationships between ambient or spike Hg concentrations and TSe concentrations in filtered water, the significance of these relationships, and the range of ambient and spike Hg concentrations. Where applicable, we report the percentage of THg that occurred as MeHg for spike and ambient THg. We also report the distribution of spike THg by total mass in abiotic compartments, and we report water chemistry variables in each mesocosm and Lake 239 (Table 1). Note that concentrations of chlorophyll a (μg/L) and dissolved organic carbon were higher in the two mesocosms at the east end next to the inflow (Treatments 0.2 and 0.4).

Concentrations of ambient THg in particulate matter were similar among mesocosms before treatment addition. After treatment addition, ambient THg concentrations in filtered water increased and then decreased below background concentrations, as did ambient THg concentrations in particulate matter. Concentrations of ambient MeHg in filtered water increased after treatment addition and then decreased to concentrations above or similar to background concentrations. In surface sediment, concentrations of ambient THg and MeHg were similar at each site before and after the experiment.

Se concentrations: temporal dynamics and effect of Se dose

Filtered Water

Before Se additions, background TSe concentrations in filtered water were similar among mesocosms at 0.15 μg/L (Figure 1). Four and eight weeks after Se addition, TSe concentrations in filtered water in each mesocosm were close to intended doses (July: r^2=0.95; p=0.006; August: r^2=0.79; p=0.11; Mean: r^2=0.98; p<0.0001).

Concentrations of Se^{4+} and TSe in filtered water were not significantly correlated (July: r^2=0.55; p=0.09; August: r^2=0.24; p=0.40; Figure 2). In filtered water, concentrations of Se^{4+} from July to August did not change except in the highest Se treatment where it decreased to one sixth. Concentrations generally ranged from 0 to 0.03 μg/L. The percentage of Se^{4+} in filtered water decreased throughout the summer. The percentage of TSe that occurred as Se^{4+} was 1 to 11% in August.

Concentrations of Se^{6+} in filtered water were significantly related to concentrations of TSe in filtered water in July (r^2=0.94; p=0.002), but not in August (r^2=0.31; p=0.25; Figure 3). Concentrations of Se^{6+} in filtered water in July were 0.01 to 0.04 μg/L and these increased in August to 0.03 to 0.06 μg/L. In August in the three lowest treatments about 15% of the TSe occurred as Se^{6+} while in the three highest treatments 5 to 7% of the TSe occurred as Se^{6+}.

Particulate Matter

Concentrations of TSe and Se^{4+} on particulate matter collected in August were significantly positively correlated with TSe concentrations in filtered water (r^2=0.88, p=0.006, $\log(TSe_{Particulate})=\log(-1.84)+1.59*\log(TSe_{Water})$; r^2=0.90, p=0.004, $\log(Se^{4+}{}_{Particulate})=\log(-1.94)+1.54*\log(TSe_{Water})$), but there was no significant relationship between concentrations of Se^{6+} on particulate matter and TSe concentrations in filtered water (r^2=0.007, p=0.87). Concentrations of TSe on particulate matter ranged from 0.001 to 0.2 μg/g of C (Figure 4). Concentrations of Se^{4+} associated with particulate matter ranged from 0.001 to 0.02 μg/g of C (Figure 5) and concentrations of Se^{6+} ranged from 0.009 to 0.012 μg/g of C (Figure 6). These values for Se species should be treated with caution because the sum of concentrations of Se^{4+} and Se^{6+} exceed the concentration of TSe.

Surface Sediment

In August, TSe, Se^{4+}, and Se^{6+} in surface sediment were not related to TSe concentrations in filtered water (r^2=0.42, p=0.17; r^2=0.43, p=0.15; r^2=0.67, p=0.18; respectively;

Figure 7). Mean concentrations ranged from 5.2 to 15.4 μg/g of TSe and 5.2 to 14.6 μg/g of Se^{4+}. Concentrations of Se^{6+} in surface sediment were near 0 to 0.4 μg/g. Eighty-nine to 105% of the TSe in surface sediment was present as Se^{4+} and 0 to 9% was present as Se^{6+}.

Did Se additions affect Hg concentrations?

Filtered Water

Spike THg was detected in samples of filtered water from all mesocosms four and eight weeks after treatment addition in July and August, respectively (Figure 8). Between four and eight weeks, ambient and spike THg declined by three to four times. There were no significant relationships between ambient THg concentrations and TSe concentrations in filtered water (July: r^2=0.02, p=0.78; August: r^2=0.01, p=0.82). There were also no significant relationships between concentrations of spike THg and concentrations of TSe in filtered water on either date (July: r^2=0.13, p=0.48; August: r^2=0.05, p=0.68). In August, concentrations of ambient THg ranged from 14 to 22 ng/L in July and 2.7 to 4 ng/L and spike THg concentrations ranged from 3 to 5 in July and 1.3 to 1.7 ng/L.

Spike MeHg was also present in filtered water throughout our experiment (Figure 9). In August, ambient MeHg concentrations in filtered water were significantly related to TSe concentrations in filtered water (August: r^2=0.89, p=0.004), but not in July (r^2=0.38, p=0.19). There were no significant relationships between concentrations of spike MeHg and TSe in filtered water in July or August (July: r^2=0.08, p=0.6; August: r^2=0.12, p=0.51). Concentrations of spike MeHg and THg in filtered water were similar in July and August. The range of ambient MeHg concentrations was 0.08 to 0.17 ng/L in July and 0.04 to 0.11 ng/L in August and the range of spike MeHg concentrations was 0.001 to 0.02 ng/L in July and August.

The percent of THg that was ambient MeHg in filtered water samples in July was twice as high in higher Se treatments as in lower Se treatments (Figure 10). The percent of spike MeHg in filtered water was four times greater in August, probably due to losses of inorganic spike Hg from water. The percent of spike MeHg ranged from 0.025 to 0.2% in July and 0.2 to 0.8% in August. In August, the range of the mass of spike THg that occurred as spike MeHg in filtered water was 0.2 to 0.12% (mean and one standard deviation: 0.05% +/- 0.04).

Particulate Matter

Concentrations of ambient THg in particulate matter were unrelated to TSe concentrations in filtered water in July (r^2=0.15, p=0.44), but in August they were significantly

positively related (r^2=0.79, p=0.02, $\log(THg_{Particulate})=\log(-0.35)+0.27*\log(TSe_{Water})$; Figure 11). There were no significant relationships between spike THg concentrations in particulate matter and concentrations of TSe in filtered water (July: r^2=0.18, p=0.41; August: r^2=0.40, p=0.18). Concentrations of spike THg in particulate matter decreased by 4 to 20 times between July and August sample dates. In July, concentrations of ambient THg were 1.4 to 17.2 ng/g of C and spike THg were 5.8 to 31.4 ng/g of C. In August, ambient THg concentrations in particulate matter ranged from 0.28 to 0.53 ng/g of C and spike THg concentrations ranged from 1.2 to 2.1 ng/g of C.

Concentrations of MeHg were also measured in particulate matter in August (Figure 12). Ambient MeHg concentrations in particulate matter showed no significant relationship with TSe concentrations in filtered water (August: r^2=0.07, p=0.61), nor did spike MeHg concentrations (August: r^2=0.19, p=0.38). In particulate matter, the range of ambient MeHg concentrations was from 0.015 to 0.021 ng/g of C and of spike MeHg concentrations was from 0.0002 to 0.0024 ng/g of C. The percentage of THg associated with particulate matter that occurred as MeHg was greater for ambient Hg than for spike Hg (Figure 13). In August the mass of spike MeHg associated with particulate matter accounted for 0.0007 to 0.007% (0.002% +/- 0.002) of the mass of spike THg added.

Surface Sediment

After eight weeks, there was no significant relationship between ambient THg concentrations in surface sediment and TSe concentrations in filtered water (August: r^2=0.04, p=0.71; Figure 14). There was also no significant relationship between spike THg concentrations in surface sediment and concentrations of TSe in filtered water (August: r^2=0.95, p=0.55). The treatment with the highest Se concentrations, Treatment 1.6, had spike THg concentrations in surface sediment similar to those of the control. Ambient THg mean concentrations in surface sediment ranged from 378.4 to 665.1 ng/g of C (d.w) while mean spike THg concentrations ranged from 74.3 to 331.3 ng/g of C (d.w.).

Spike MeHg was also detected in surface sediment in August (Figure 15). Ambient MeHg concentrations in surface sediment had no significant relationship with TSe concentrations in filtered water (August: r^2=0.05, p=0.65). Spike MeHg concentrations in sediment also were not significantly related to TSe concentrations in filtered water (August: r^2=0.01, p=0.84). The range of ambient MeHg concentrations was from 15.0 to 35.8 ng/g of C (d.w.) and of spike MeHg concentrations was from 0.4 to 4.8 ng/g of C (d.w.). The percentage

of ambient THg in surface sediment that occurred as MeHg was greater than that of spike THg (Figure 16). The percentage of spike THg that occurred as spike MeHg in surface sediment ranged from 0.4 to 1.4% while that of ambient MeHg ranged from 3.5 to 5.2%. Surface sediment contained a greater percentage of spike MeHg than filtered water and particulate matter and accounted for 0.08 to 1.0% (0.33% +/- 0.33) of the mass of spike THg added.

Mass balance of spike Hg and the effect of Se loading

On the final sample date, the masses of spike THg in each compartment among mesocosms were similar (except in surface sediment in Treatment 0.8 which was 2 to 3 times greater than others). Surface sediment contained the greatest proportion of spike THg followed by filtered water and then particulate matter. Using these three compartments we accounted for 40 to 55% of the added spike Hg (95% in Treatment 0.8 because of the high mass in surface sediment; Figure 17). Concentrations of THg were elevated in two of the three samples collected from Treatment 0.8. We assumed most of the unaccounted spike THg was evaded to the atmosphere and that less than 1% was present in biota. The error terms associated with the mass of spike THg in each compartment are surface sediment (+/-366 621 ng), filtered water (+/-6 231 ng), and particulate matter (+/-28 241 ng; Table 2).

The mass of spike MeHg was greatest in surface sediment (4 922 to 58 661 ng), followed by filtered water (1 824 to 7 079 ng), and then particulate matter (12 to 404 ng). Of the entire added spike THg, the mass of spike MeHg in surface sediment, filtered water, and particulate matter accounted for less than 1.0, 0.16, and 0.007%, respectively. Therefore about 1% of the added spike THg was methylated.

The mass of TSe dissolved in water increased with Se dose but that associated with surface sediment did not (Table 3). There was more TSe in surface sediment than dissolved in water in Treatments 0, 0.1, 0.4, and 0.8 while in Treatments 0.2 and 1.6 there was more Se dissolved in water than in surface sediment. The masses of Se calculated in each mesocosm were greater than the mass of Se that was added.

DISCUSSION

Were Se concentrations or partitioning related to Se dose?

The concentrations of TSe in filtered water were close to intended doses both four and eight weeks after treatment addition. This may indicate that Se was fairly stable in the aqueous phase. We expected concentrations of Se to decrease throughout our experiment due to

adsorption to particulate matter, deposition to the surface sediment, and possibly volatilization. The relatively slow loss of Se suggests that the need for repeated additions of Se in whole lakes could be less than expected. Mesocosms may have retained Se in water, but lakes and reservoirs flush water, have variable circulation patterns, and stratify, which could all contribute to less stable Se concentraitons.

We could not calculate a half-life of added Se in our mesocosms but the half-life of Se has been described in other studies. ^{75}Se added to an ELA lake was transferred to the sediment via suspended particulate matter or adsorption (Hesslein *et al.* 1980). Fifty percent of the added ^{75}Se was detected in sediment 331 days after addition and 54% of it was detected in water. Sediment was also the major sink of ^{75}Se in mesocosms (Turner and Rudd 1983). In their control mesocosms, which included ^{75}Se but no additional Se treatment, deposition of ^{75}Se stabilized after six weeks and water concentrations of Se were also stable. In Se treatments, however, ^{75}Se remained in the ionic phase in water and therefore continued to decrease without stabilizing after six weeks. The half-life of ^{75}Se in the lake water was 49 to 52 days (Hesslein *et al.* 1980; Turner and Rudd 1983). Differences in the half-lives between our experiment and others may be due to differences in particulate concentrations, sediment to water ratio, and duration of the experiment. During installation, tree pollen at the edge of the lake was incidentally trapped in mesocosms, which could have adsorbed Hg and Se and transported it to the sediment.

Relationships between Se species concentrations and Se dose were most apparent in particulate matter. Total Se and Se^{4+} associated with particulate matter were significantly correlated with TSe concentrations in filtered water. When more Se was present in filtered water, more Se was also found in particulate matter. None of the Se species in surface sediment were related to TSe concentrations in filtered water. This may have been due to the short duration of the experiment. The process of Se transfer to the sediment probably takes longer than adsorption to suspended particulate matter. However, most of the mass of Se was present in sediment in most mesocosms. This represented ambient and added Se, so it is impossible to discern whether transfer of added Se to sediment was slower, faster, or whether Se changed its chemical form upon deposition.

Did Se additions alter Hg or MeHg concentrations in abiotic compartments?

We simulated Se additions to water bodies at different concentrations using mesocosms and added tracer Hg to provide sensitivity. Partitioning of spike Hg among filtered water,

particulate matter, and surface sediment did not differ among mesocosms. In July and August no significant relationships existed between ambient or spike THg or MeHg concentrations in filtered water and TSe concentrations in filtered water, except for a positive relationship with ambient MeHg concentrations in filtered water in August. Concentrations of ambient or spike THg and MeHg in particulate matter were not significantly related to TSe concentrations in filtered water except for a positive relationship with ambient THg concentrations on particulate matter in August. In addition, concentrations of ambient or spike THg and MeHg in surface sediment were not significantly related to TSe concentrations in filtered water. Therefore we could not reject our null hypothesis that Se additions do not affect THg or MeHg concentrations in filtered water, particulate matter, and surface sediment, aside from the exceptions that ambient MeHg concentrations in filtered water and ambient THg concentrations on particulate matter increased with increasing TSe concentrations in filtered water. We did not see a difference in the dynamics of Hg with regard to the three forms of Se that were analyzed. Cycling of Se in abiotic compartments also was apparently not the cause of modifications of Hg uptake in the food web (Chapter 4).

Previous studies that examined the response of Hg bioaccumulation with different doses of Se used higher concentrations of Se than in our experiment. In a mesocosm study in Clay Lake, Ontario, 1 to 100 μg/L of Se was used (Turner and Rudd 1983). In Swedish whole-lake studies, 2 to 5 μg/L was added to water (Paulsson and Lundbergh 1991). These experiments occurred at Se concentrations that were above the range observed to cause elevated Se concentrations in gonads of fish and reproductive toxicity (Lemly 2002b). Surveys of lakes with varying Se concentrations have usually observed lower Hg concentrations in fish when Se concentrations in water or muscle were higher (Chen *et al.* 2001; Speyer 1980; Leskinen *et al.* 1986; Mauk and Brown 2001). To examine the effects on Hg cycling, this study used Se concentrations less than 1.6 μg/L and it looked at the endpoint for chronic Se toxicity.

Timing and location of spike Hg partitioning

Spike THg was present in filtered water four weeks after treatment addition and also after eight weeks but at lower concentrations. Spike THg concentrations in particulate matter decreased from about 600 to 60 μg/g between four and eight weeks yet remained higher than ambient THg concentrations. After eight weeks, spike THg was detectable in surface sediment and it accounted for the largest pool of spike THg in these systems. After this eight week experiment, we do not expect these systems to have been at steady state with regard to Hg and

Se cycling. On the basis of mass balances after eight weeks, Se dose had no detectable effect on spike Hg partitioning.

Spike THg concentrations in filtered water declined quickly after treatment additions. The half-life of spike THg in water was 26 to 36 d. The half-life of Hg in other studies was 14.3 d (Hesslein *et al.* 1980), 16 to 36 d (Rudd *et al.* 1980), 20 to 23 d (Turner and Rudd 1983), and approximately 11 d (Orihel *et al.* 2006). In our study, the half-life of added Hg was within those ranges, but at the higher end. It was most similar to other studies that also added Hg and Se, but the half-life in Treatment 0 was also short, indicating there was no link between the half-lives of Se and Hg. The major loss mechanism of added Hg from the water column was in the unknown portion, which we assumed to be atmospheric evasion. The next most important loss mechanism was sedimentation either by deposition of particulate matter or direct adsorption.

Past mesocosm and whole lake studies have found that the major sink of Hg was either the sediments or water. This difference probably depends on the length of the experiment as most Hg would initially be in the water but would ultimately settle to the sediment. Hesslein *et al.* (1980) found sediment to be the major sink for ^{203}Hg via deposition of suspended particulate matter and adsorption in a whole lake experiment in a boreal lake, but this study did not assess evasion. In a mesocosm experiment in a turbid, boreal lake, redistribution of ^{203}Hg from water to sediment was similar at treatments of 1 and 10 µg/L of Se (20 to 23 day half-life), but at 100 µg/L of Se the ^{203}Hg had a shorter half-life (Turner and Rudd 1983). Despite similar half-lives at 1 and 10 µg/L of Se, the partitioning of ^{203}Hg differed at these different Se concentrations. In controls, 30% of added ^{203}Hg was extracted when passed through a charcoal filter whereas in Se treatments 23% of the ^{203}Hg was associated with this compartment. Hg extracted with the charcoal filter represents the Hg complexed with organic matter or metals. Therefore Se lowered the amount of Hg associated with organic matter, but did not affect the partitioning of ^{203}Hg among particulate, ionic, and dissolved fractions (Turner and Rudd 1983). More recent mesocosm studies did not find sediment to be the major sink of added Hg. Ten to 12% of spike Hg was found in water whereas about 4% was found in sediment in a mesocosm experiment in a boreal lake (Paterson *et al.* 2006). In a different mesocosm experiment, a maximum of 16% of the spike Hg was associated with particulate matter whereas 8 to 16% was associated with sediments (Orihel *et al.* 2006). In both of these mesocosm studies, the relative importance of the sinks were the atmosphere, water, and then sediment (Paterson *et al.* 2006; Orihel *et al.* 2006).

The entire mass of spike THg added to mesocosms was not accounted for in the mass balance. Atmospheric evasion of Hg and Se were not measured and thus the largest amount of

Hg lost was calculated by difference (7 to 63%). Treatment 0.8 had an abnormally large amount of spike Hg in two of the three randomly collected sediment samples. The amount of Hg we assumed to be lost by evasion was within the range of those measured in other studies: 33 to 59% (Poulain *et al.* 2006) and 36% per 30 days (Amyot *et al.* 2004). Evasion was not estimated in older studies.

Spike MeHg formation

Spike MeHg was produced and bioaccumulated within the first four weeks of our experiment (Chapter 5). The largest amount of spike MeHg was in the surface sediment and we assume this to be the most important site of Hg methylation. The percentage of spike Hg that was methylated and found in abiotic compartments ranged from 0.1 to 1.2% and sediment contained 0.4 to 4.8 ng/g (d.w.) of spike MeHg. Again, this percentage of methylated spike Hg probably does not represent steady state conditions. In another mesocosm experiment in a boreal lake, less than 1% of the added isotopic Hg was converted to MeHg after eight weeks and sediment contained 0.2 to 11 μg/g of Hg at similar loading rates (Orihel *et al.* 2006). Further, in our study the significant relationship between ambient MeHg concentrations in filtered water and concentrations of TSe in filtered water may indicate that low concentrations of Se enhance MeHg production as reported previously (Jackson 1991; Chen *et al.* 1997; Jin et al. 1999). If Se enhances MeHg concentrations, one would expect there to be more MeHg available to biota. This has the potential to result in greater MeHg uptake in the food web in systems with Se concentrations that optimize Hg methylation.

Do mesocosms approximate larger systems?

There are benefits and limitations to using mesocosms. Replicated experimental units increase statistical credibility and randomization lowers biases due to spatial variation or sampling procedures (Hurlbert 1984) and provide greater control over treatments. Reasonable similarities between microcosms and natural systems with regard to Hg cycling have been observed (Saouter *et al.* 1995). There was little deviation between our control mesocosm and the host lake for measures of water chemistry variables (Table 1).

One dose of Hg simulated a pulse of Hg that could be released in new reservoirs. Much of the Hg released in new reservoirs could be MeHg rather than inorganic Hg. In addition, annual Hg deposition is greatest in the warm season (Mason *et al.* 2000), similar to our experiment. The single dose of Se best simulates how Se would be added if it were applied to

aquatic systems to lower Hg. The long residence time of Se in water also supports adding a single dose as multiple doses did not seem necessary to maintain target Se concentrations during this two-month study.

A drawback of mesocosms is their inability to represent the complexity of large systems. For example, mesocosms do not include interactions between the littoral and pelagic zones or the catchment and the lake (Schindler 1998). Our mesocosms did not stratify, had no circulation with the open lake, and excluded top predators. Short experiments do not allow for equilibrium conditions or responses from organisms that are slow to respond (Harrison *et al.* 1990; Schindler 1998; Paterson *et al.* 2006). Mesocosms also exclude top predators because small closed systems cannot support them (Schindler 1998). Also, population dynamics and behaviours may be altered in smaller organisms. The large surface area supports more periphyton growth than would be naturally possible. For each mesocosm the financial and man-power expenses can be similar to a whole-lake experiment. Despite these inadequacies, using longer term mesocosm studies could provide more realistic expectations for applying Se additions to aquatic systems.

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Table 1. Water chemistry variables measured from the centre location and depth of each mesocosm and from the epilimnion of Lake 239. The values are the mean of four sample dates.

Mesocosm	**1**	**2**	**3**	**4**	**5**	**6**	**Lake 239**
Treatment (µg/L of Se)	**0.1**	**1.6**	**0**	**0.8**	**0.2**	**0.4**	
Total Dissolved P (µg/L)	3.3	3.7	2.7	5.3	3.3	3.3	2.5
Suspended P (µg/L)	6.3	4.0	4.3	4.0	4.0	4.3	4.0
Total Dissolved N (µg/L)	288.7	282.0	278.0	378.7	303.3	301.0	256.0
Ammonium (µg/L)	13.3	10.3	25.0	16.7	10.5	9.5	12.0
Dissolved Organic C (µM/L)	661.0	642.7	685.3	643.3	706.0	749.0	635.5
Chlorophyl *a* (µg/L)	0.8	0.9	0.8	0.7	1.5	2.4	1.9
pH	7.4	7.4	7.5	7.3	7.3	7.2	7.4
Sediment (% C)	0.004	0.003	0.004	0.003	0.003	0.01	

Table 2. Mass balance of spike THg in surface sediment, filtered water, and particulate matter. The mass found in each mesocosm after eight weeks was subtracted from the mass of spike Hg added to each mesocosm. The error terms were calculated using only concentrations (not including error terms associated with water volume, sediment area, and sediment density) and therefore they are underestimates.

Mesocosm	**3**	**1**	**5**	**6**	**4**	**2**
Treatment (Se µg)	**0**	**0.1**	**0.2**	**0.4**	**0.8**	**1.6**
Mass spike THg added (ng)	5850000	5850000	5850000	5850000	5850000	5850000
Sediment (ng)	1498876	2088958	1823149	1294451	4408400	1309647
SD	746744	366621	215848	280303	2494510	609106
Water (ng)	812636	909874	697209	852698	866149	720544
SD		6231				
Particulate (ng)	98762	158248	82052	220047	112886	132599
SD		28241				
Difference in mass of spike THg measured and that added as treatments (ng)	-3439726	-2692921	-3247590	-3482804	-462566	-3687211
Sediment (%)	26	36	31	22	75	22
Water (%)	14	16	12	15	15	12
Particulate (%)	2	3	1	4	2	2
Unknown (%)	59	46	56	60	8	63

Table 3. Masses of Se added to mesocosms as treatments and Se found in filtered water, particulate matter, and surface sediment. Mesocosms contained a significant amount of Se before treatment addition. The error terms were calculated using only concentrations (not including error terms associated with water volume, sediment area, and sediment density) and therefore they are under estimates.

Treatment (Se µg/L)	**0**	**0.1**	**0.2**	**0.4**	**0.8**	**1.6**
Se Added (µg)	0	19635	39270	78540	157080	314160
Sediment (µg)	150608	129555	99590	207715	300419	141330
SD	125943	60703	45068	9582	148704	87791
Water (µg)	69198	74147	155133	188596		261675
SD		1750				
Particulate (µg)	83	154	259	1274	1161	899
SD		177				
Mass of Se measured minus that added as treatments (µg)	219889	184220	215712	319044		89743
Sediment (%)	0	660	254	264	191	45
Water (%)	0	378	395	240		83
Particulate (%)	0	0.8	0.7	1.6	0.7	0.3

FIGURES

Figure 1. Concentrations of TSe in filtered water as a function of intended Se dose in water in June (black circles), prior to Se additions, July (triangles), and August (squares). Each data point represents one sample. The regression line is for July data. The variable x is the intended Se dose and y is the measured TSe concentration.

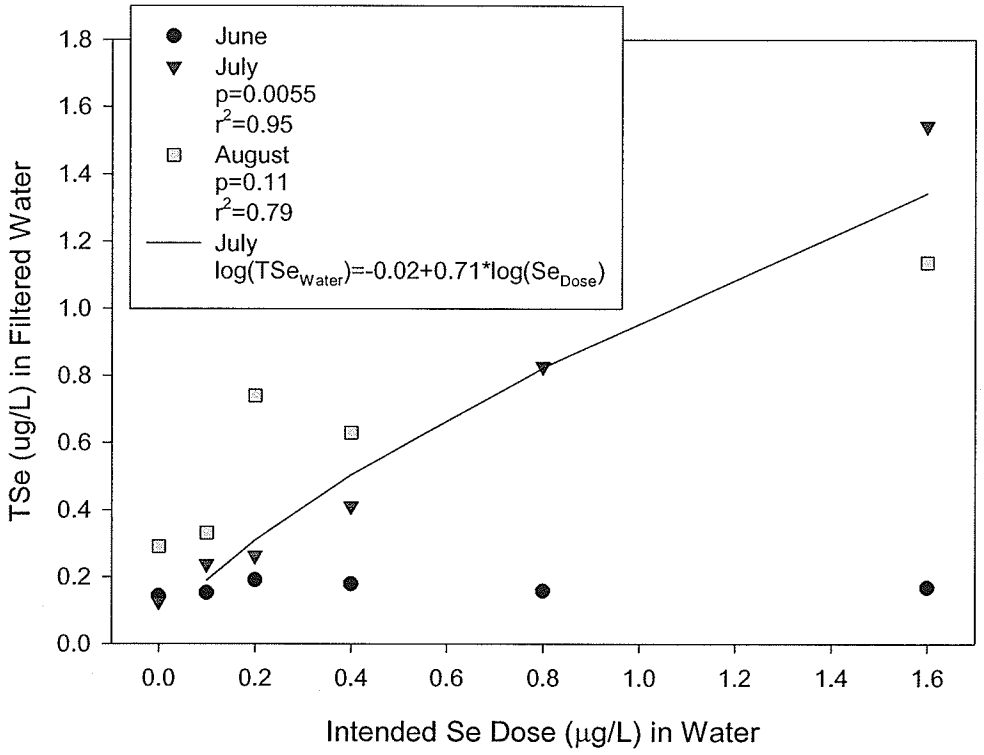


Figure 2. Concentrations of Se^{4+} in filtered water as a function of TSe concentrations in filtered water in June (black circles), July (triangles), and August (squares). Each data point represents one sample.

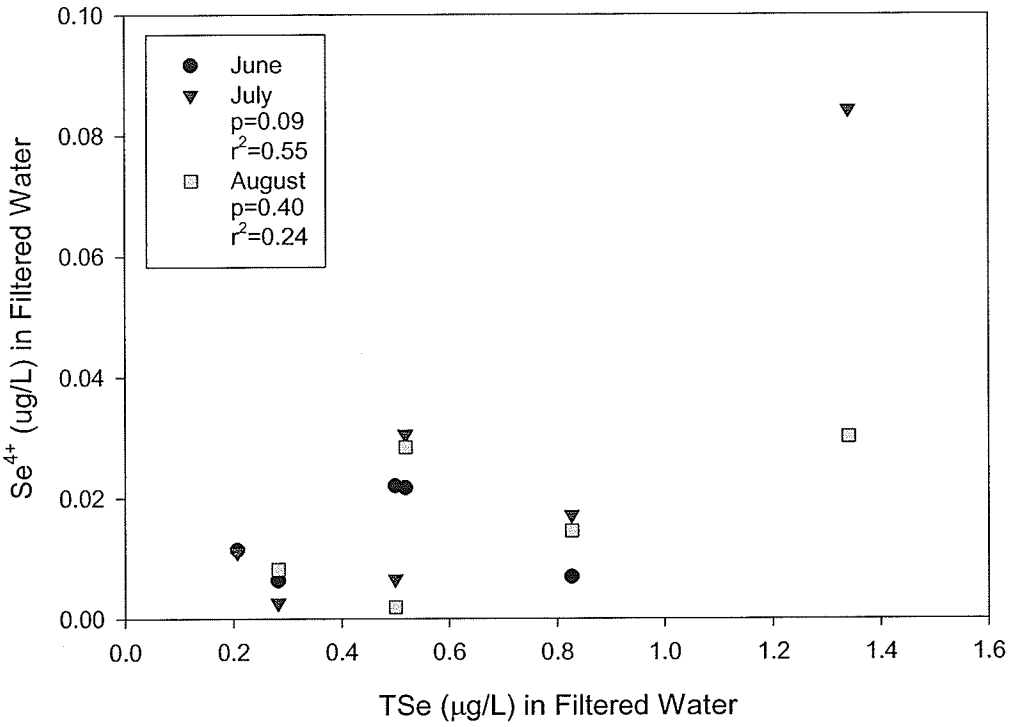


Figure 3. Concentrations of Se^{6+} in filtered water as a function of TSe concentrations in filtered water in June (black circles), July (triangles), and August (squares). Each data point represents one sample. The regression line is for July data. The variable x is the TSe concentration in water and y is the Se^{6+} concentration in water.

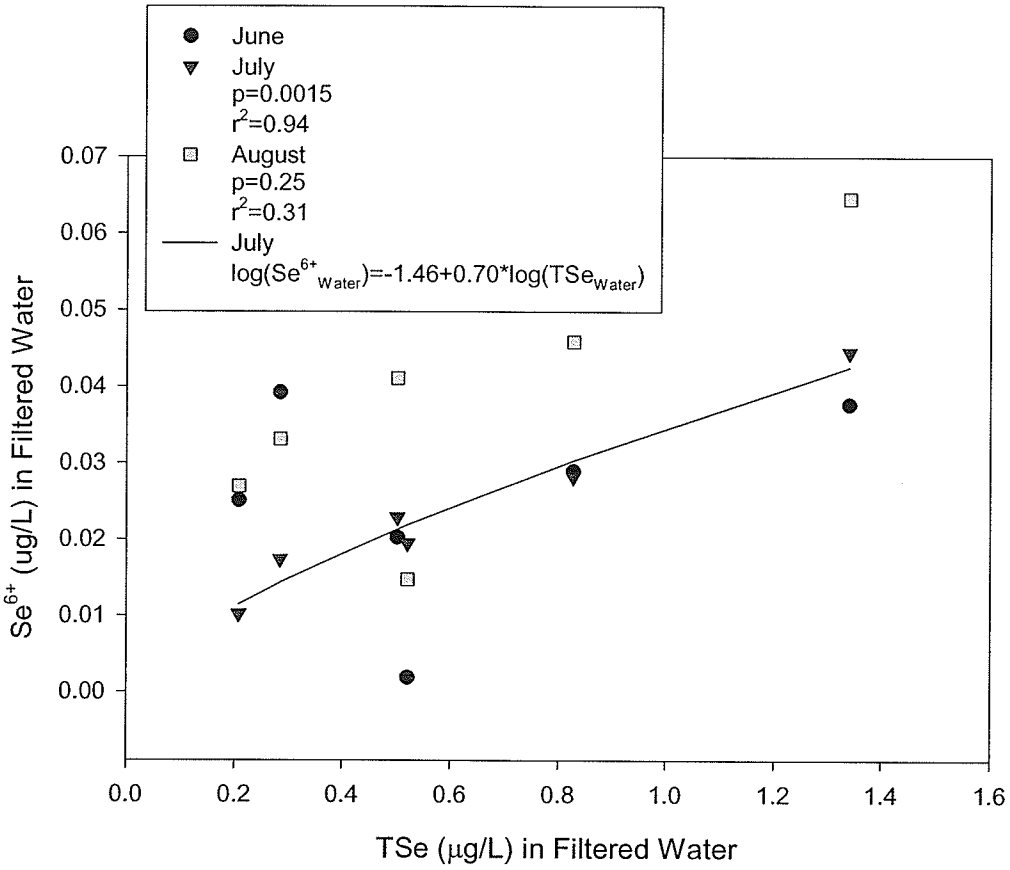


Figure 4. Concentrations of TSe in particulate matter as a function of TSe concentrations in filtered water in August. Each data point represents one sample. The variable x is the TSe concentration in water and y is the TSe concentration in particulate matter.

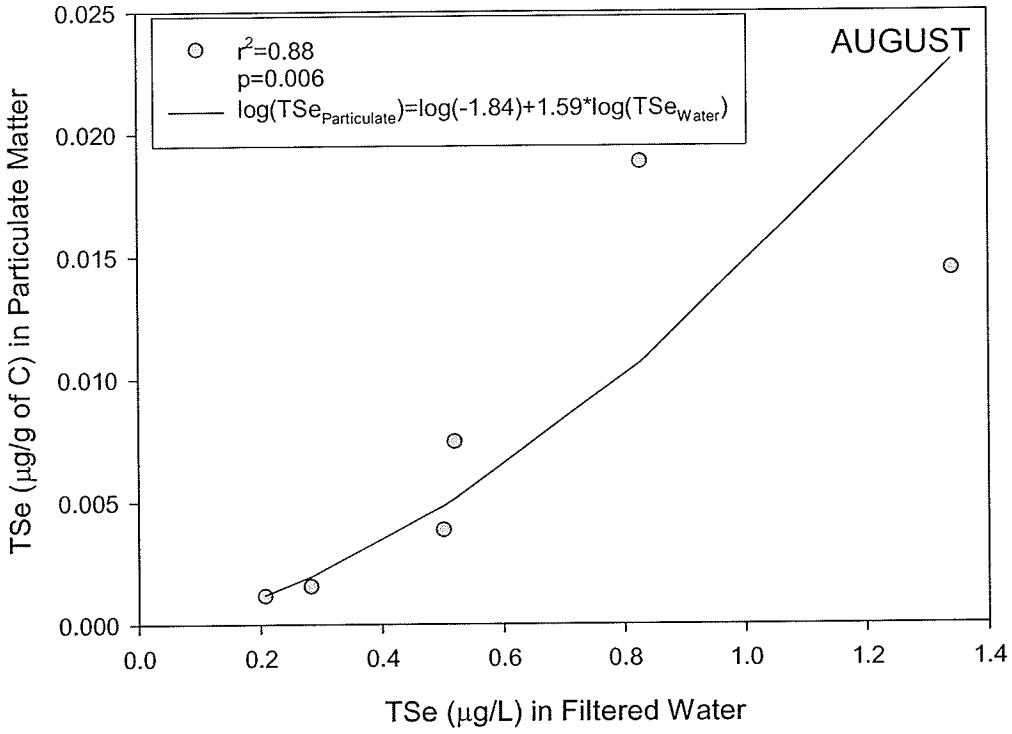


Figure 5. Concentrations of Se^{4+} in particulate matter as a function of TSe concentrations in filtered water in August. Each data point represents one sample. The variable x is the TSe concentration in water and y is the Se^{4+} concentration in particulate matter.

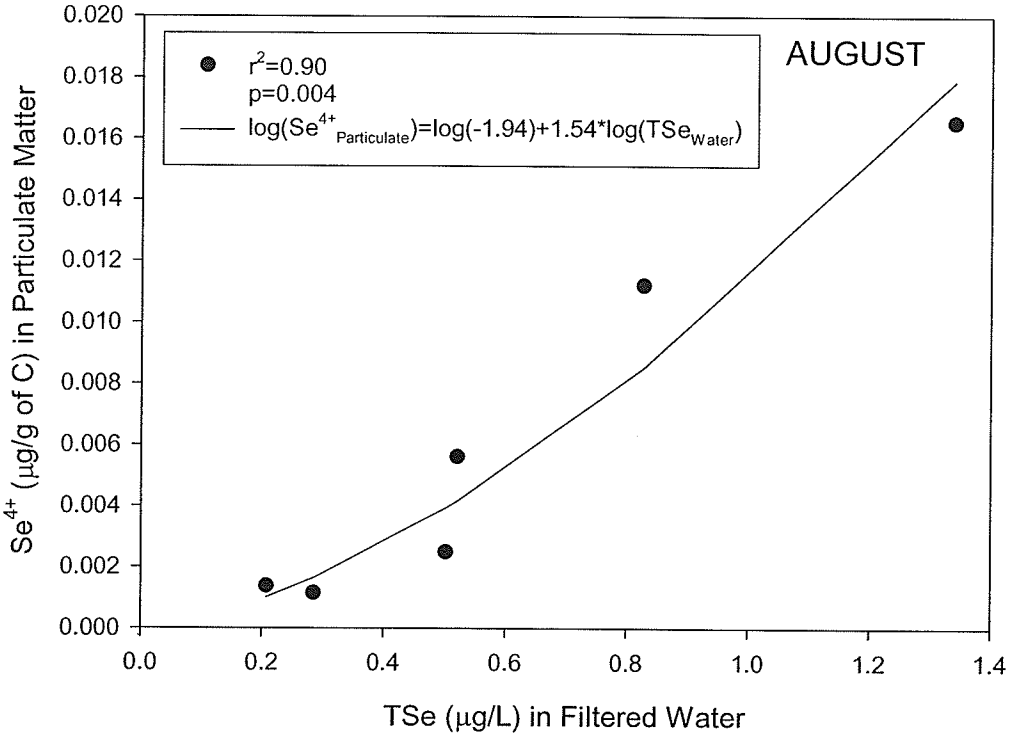


Figure 6. Concentrations of Se^{6+} in particulate matter as a function of TSe concentrations in filtered water in August. Each data point represents one sample. The variable x is the TSe concentration in water and y is the Se^{6+} concentration in particulate matter.

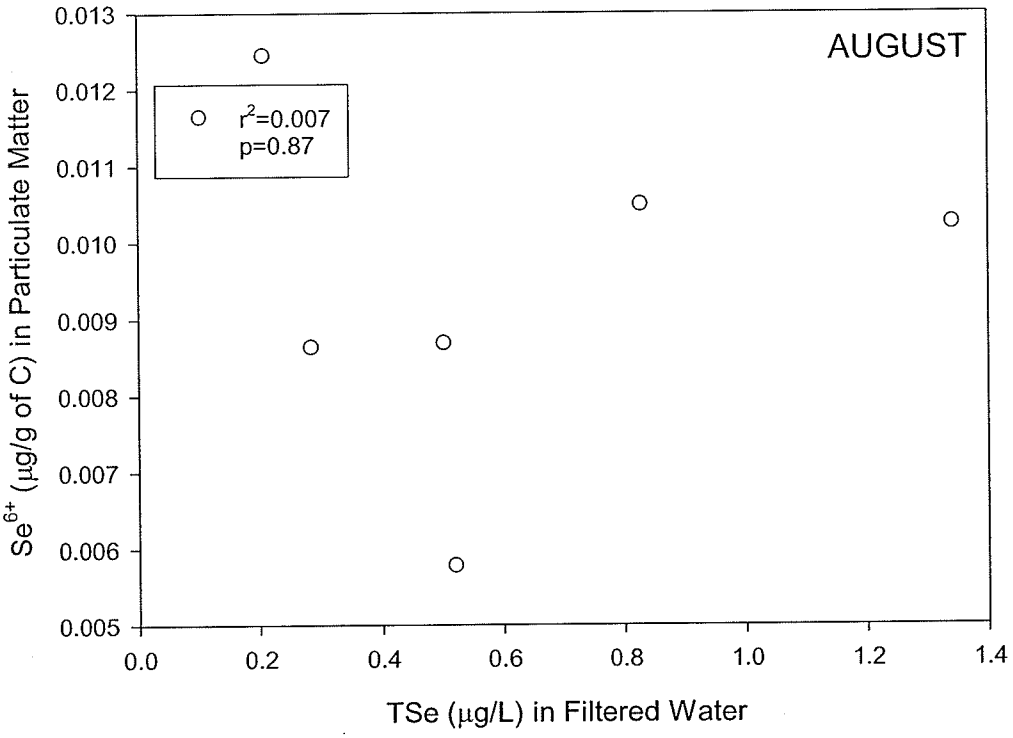


Figure 7. Concentrations of TSe, Se^{4+}, and Se^{6+} in surface sediment as a function of TSe concentrations in filtered water. Each data point represents the mean of three samples of sediment with one standard deviation.

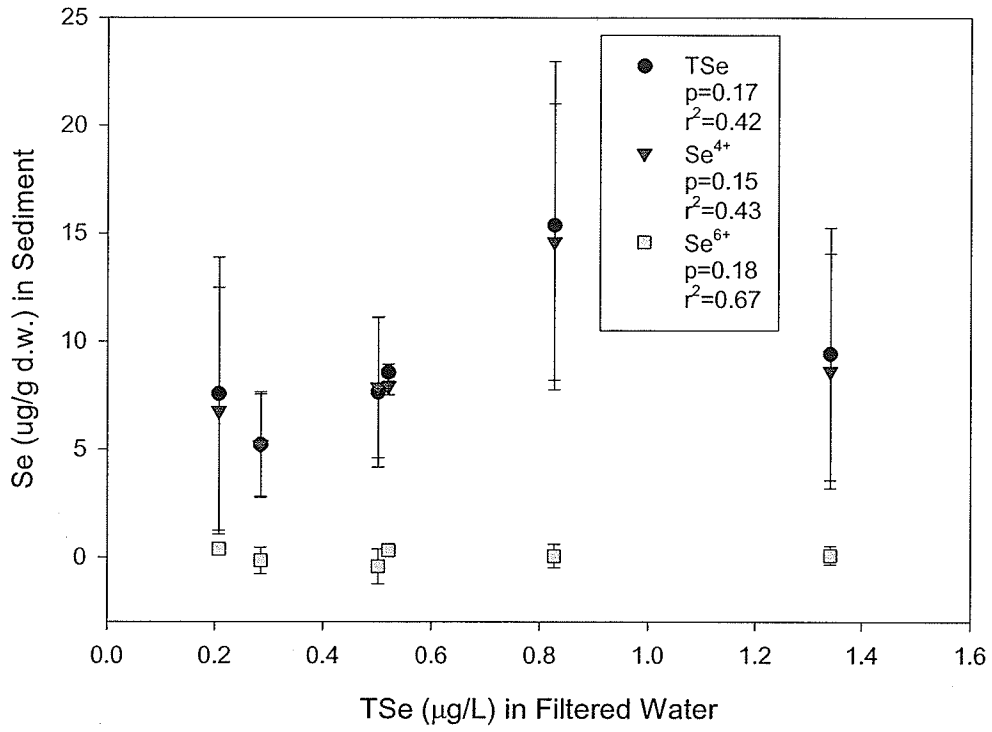
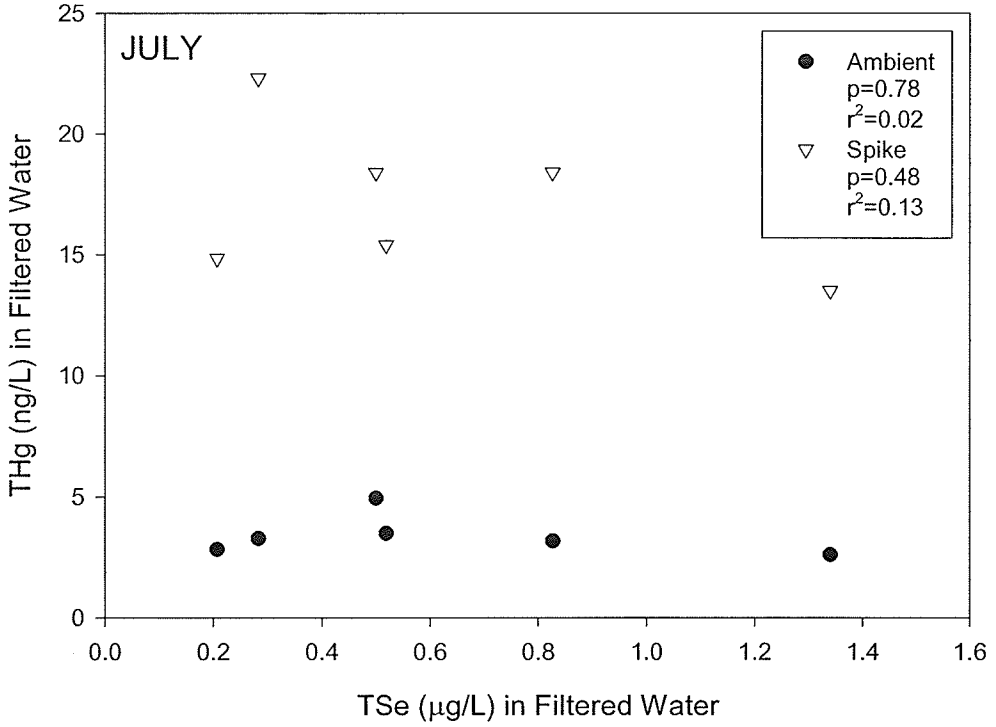


Figure 8. Ambient and spike THg concentrations in filtered water as a function of TSe concentrations in filtered water. Each data point represents one sample.



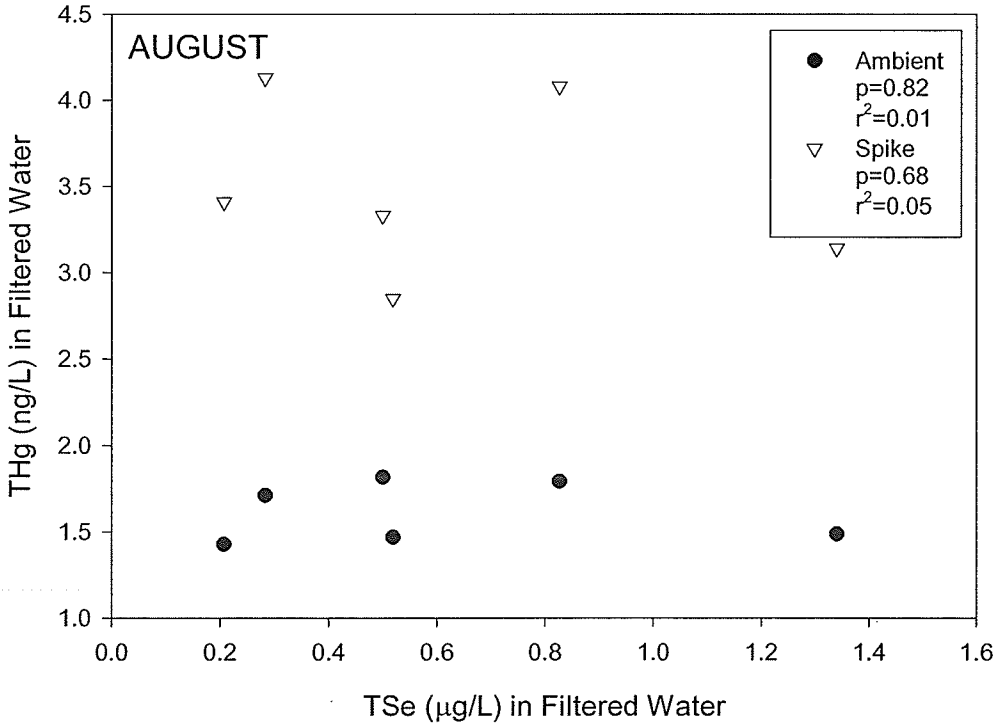
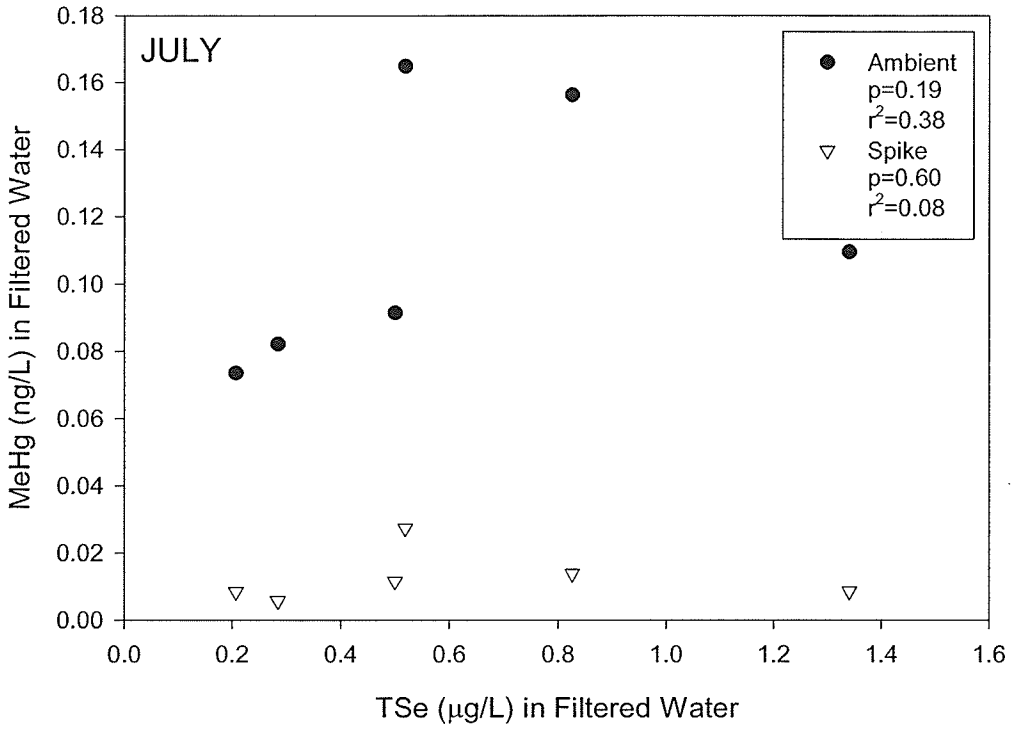


Figure 9. Ambient and spike MeHg concentrations in filtered water as a function of TSe concentrations in filtered water. Each data point represents one sample.



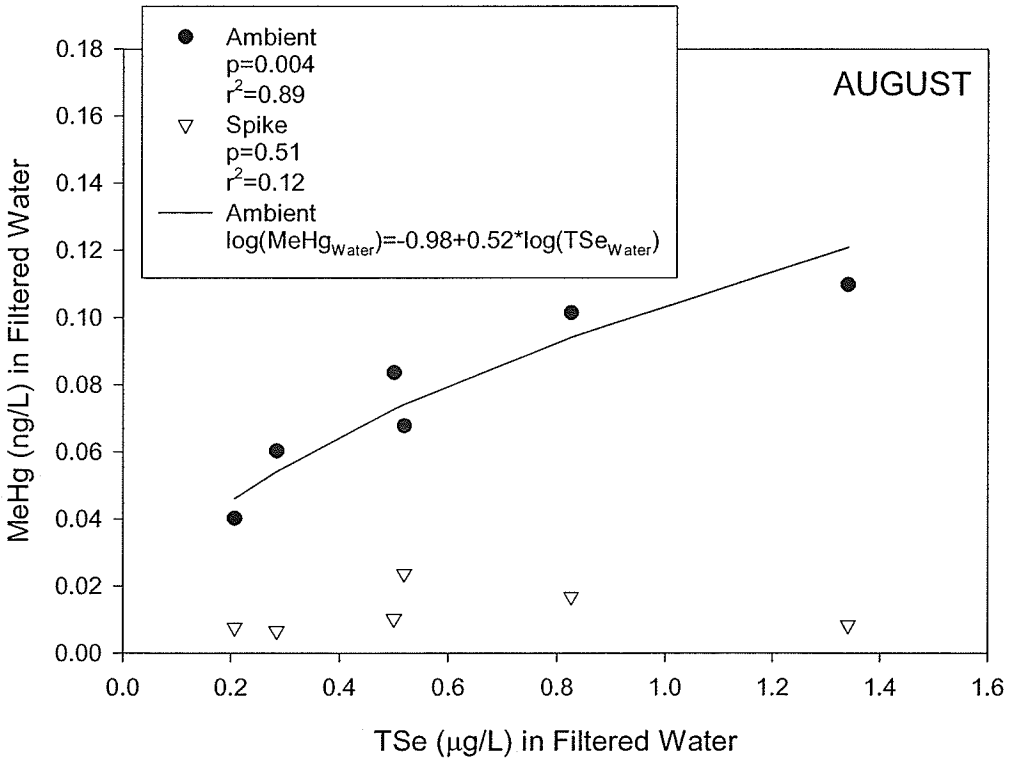
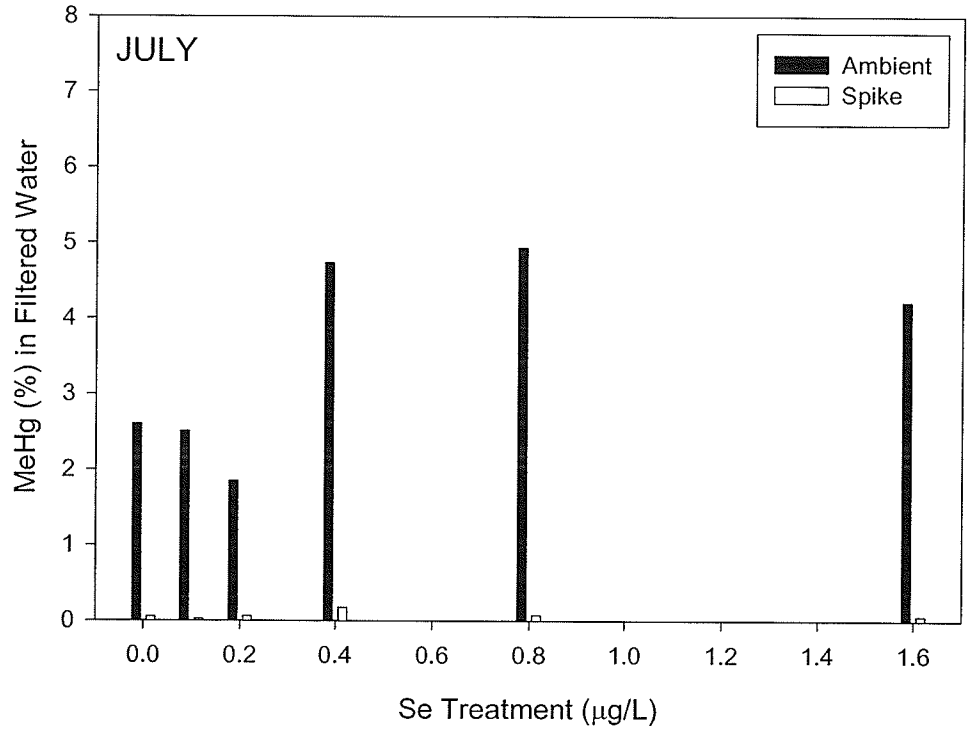


Figure 10. Percentages of THg that occurred as MeHg in filtered water of each treatment. Each bar represents one sample.



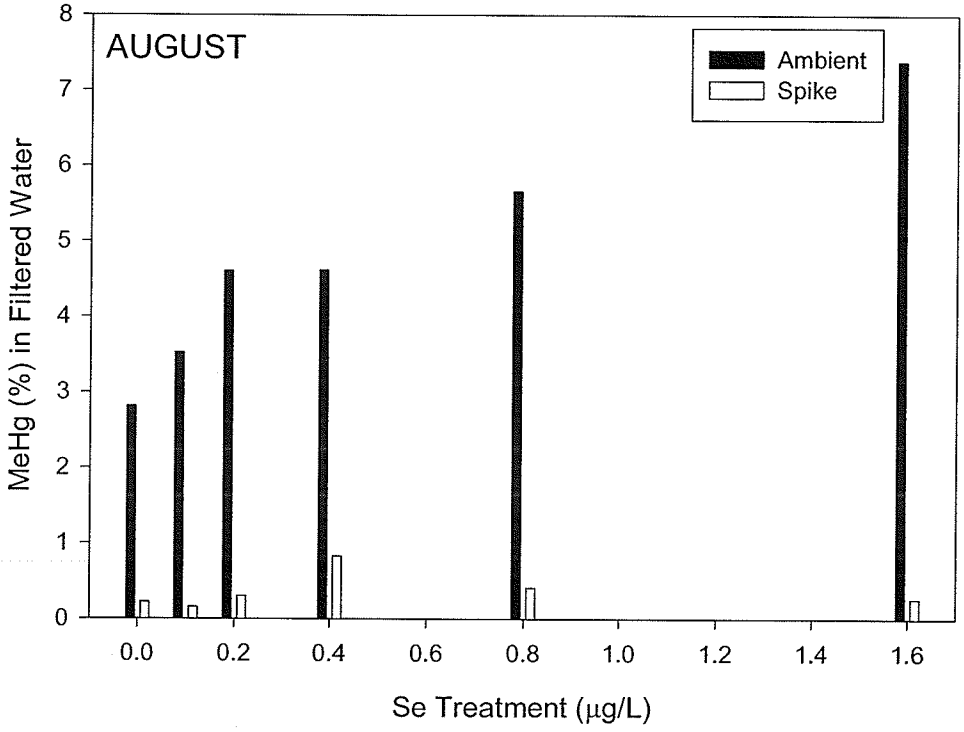


Figure 11. Ambient and spike THg concentrations in particulate matter as a function of TSe concentrations in filtered water. Each data point represents one sample.

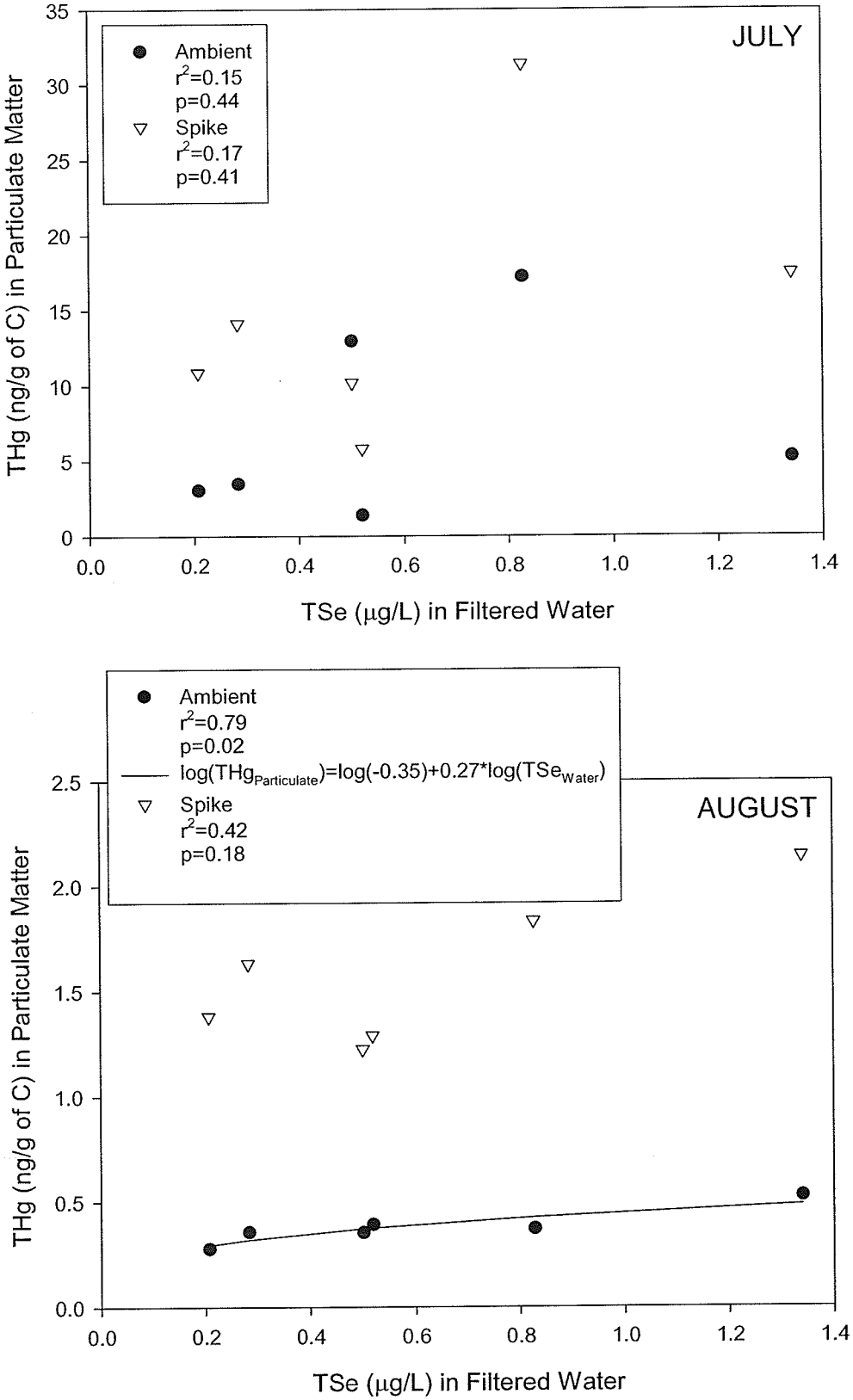


Figure 12: Ambient and spike MeHg concentrations in particulate matter as a function of TSe concentrations filtered water. Each data point represents one sample.

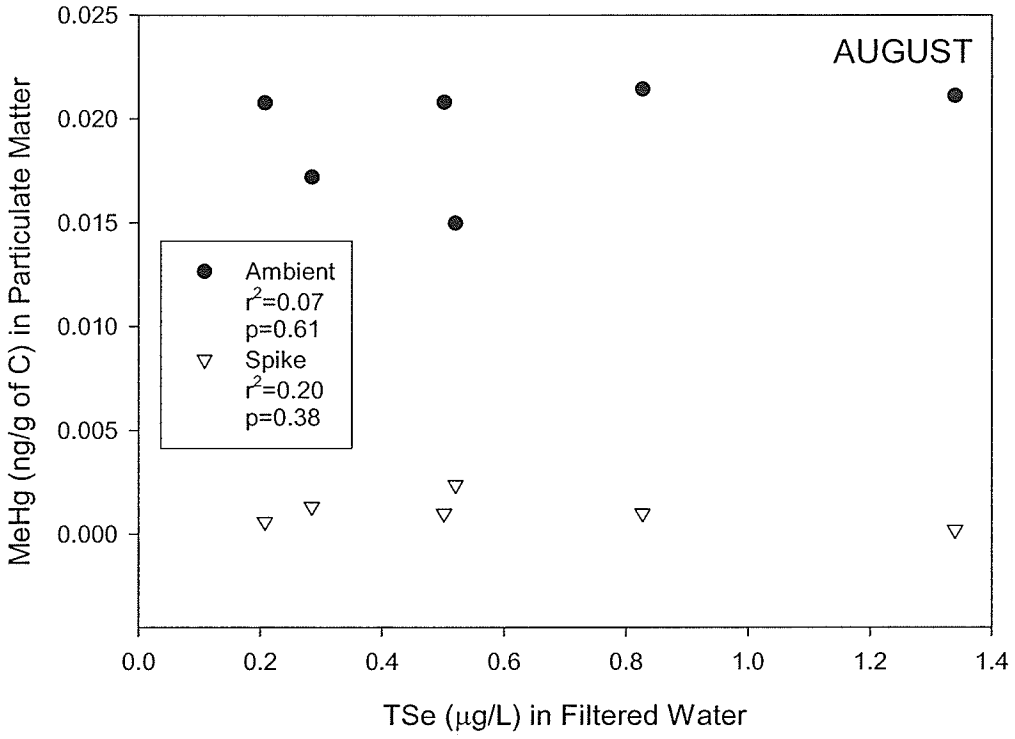


Figure 13. Percentages of THg that occurred as MeHg in particulate matter of each treatment. Each bar represents one sample.

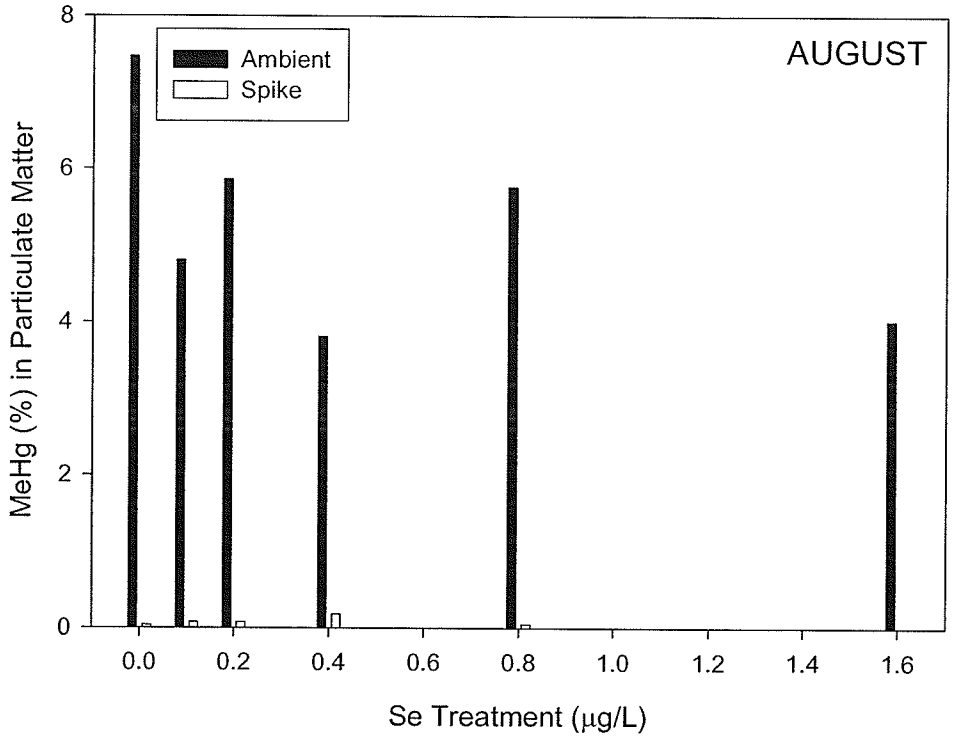


Figure 14. Ambient and spike THg concentrations in surface sediment as a function of TSe concentrations in filtered water. Each data point represents the mean of three samples of surface sediment with one standard deviation.

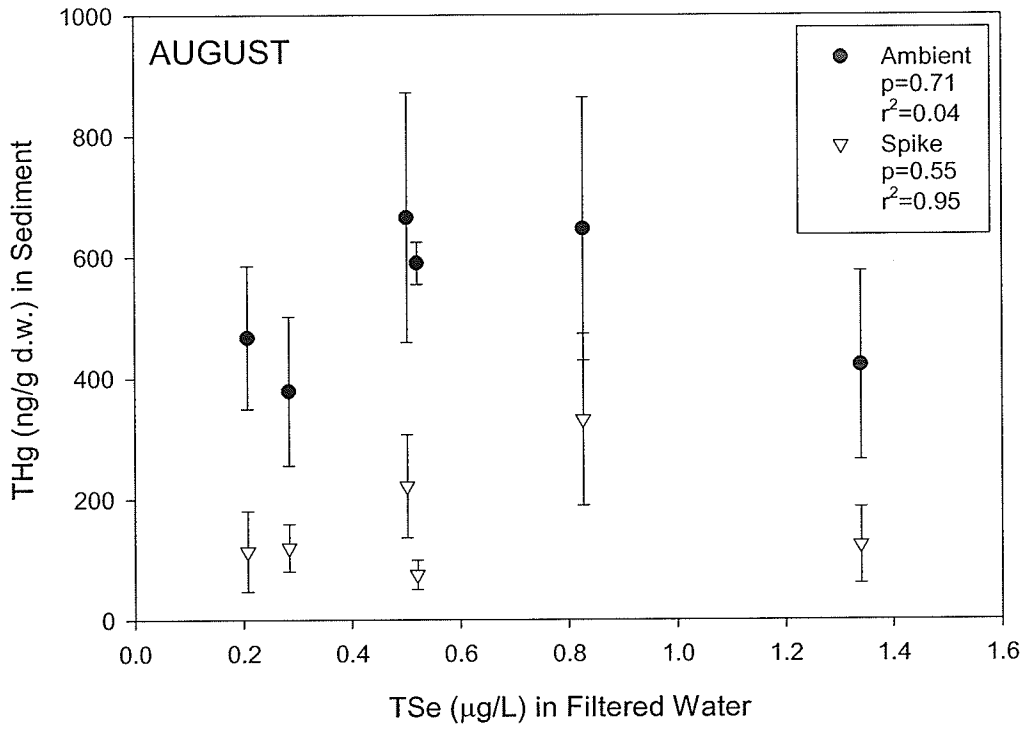


Figure 15. Ambient and spike MeHg concentrations in surface sediment as a function of TSe concentrations in filtered water. Each data point represents the mean of three samples of surface sediment with one standard deviation.

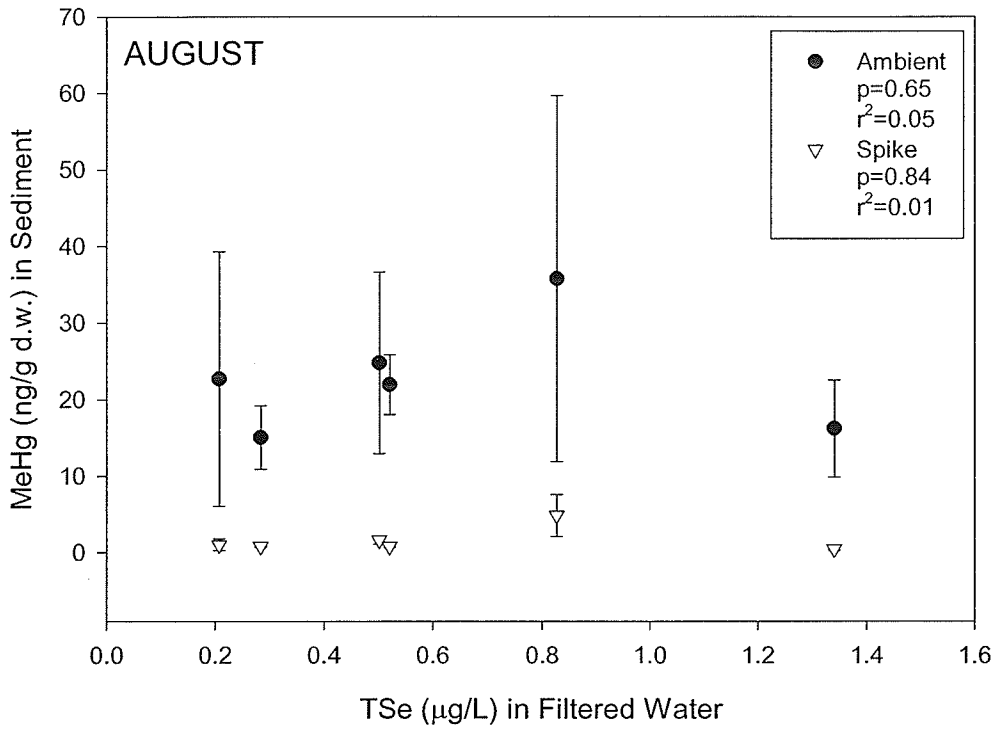


Figure 16. Percentages of THg that occurred as MeHg in surface sediment of each treatment. Each bar represents the mean of three samples of surface sediment with one standard deviation.

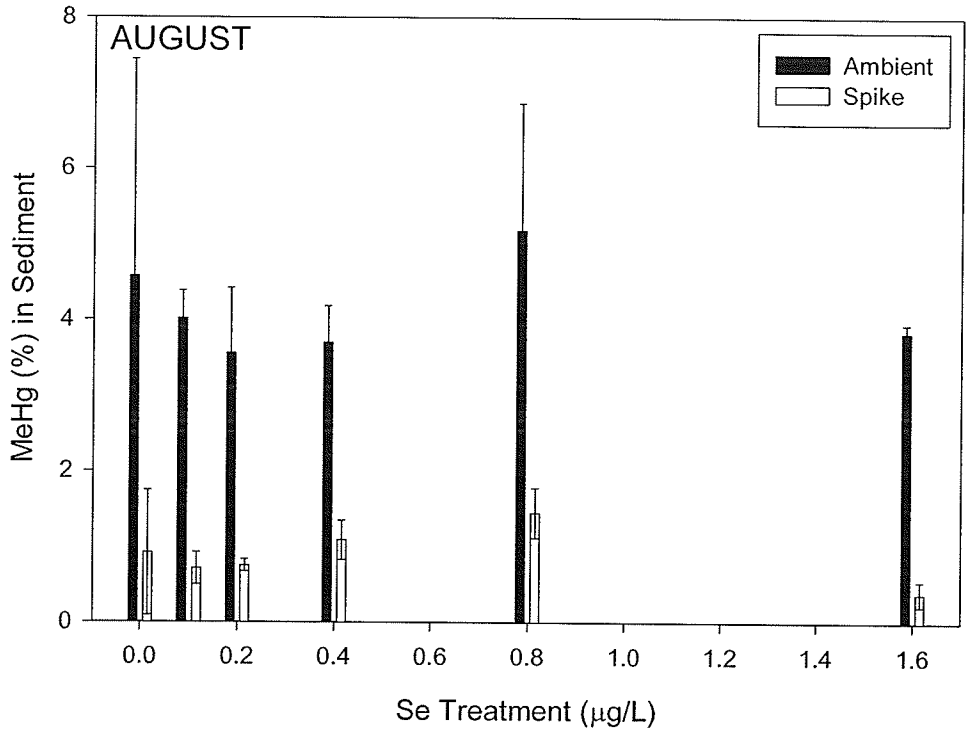
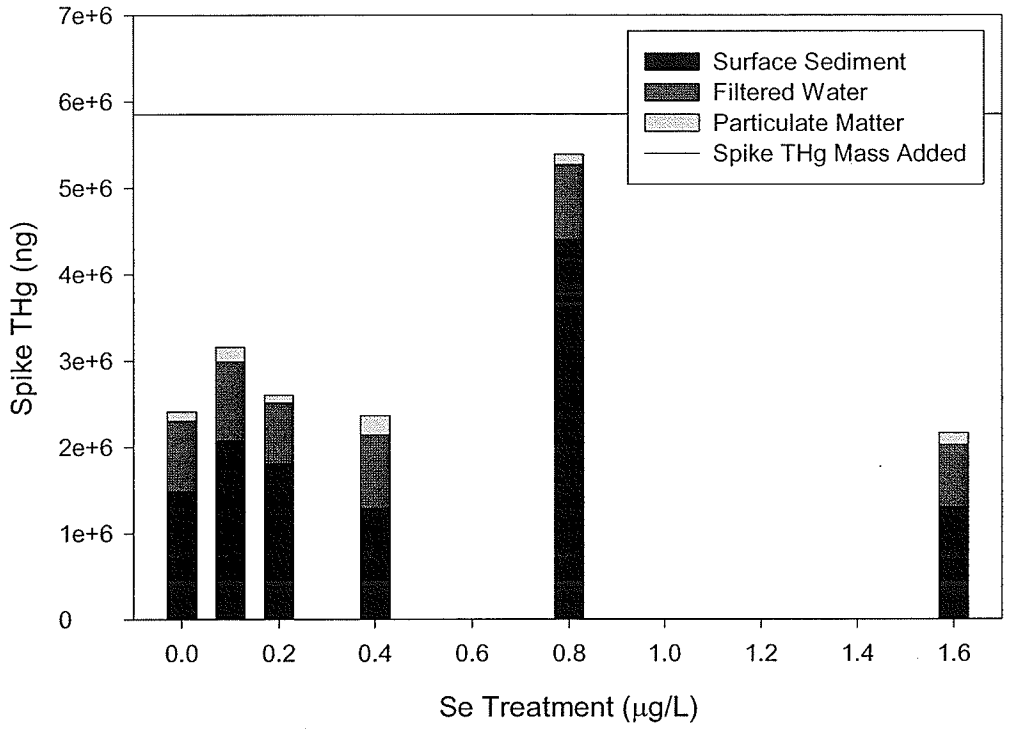


Figure 17. Masses of added spike THg in surface sediment, filtered water, and particulate matter in August. The mass of spike THg added to each mesocosm was 5.85 mg. Each bar for particulate matter and filtered water represents one sample while that for surface sediment represents the mean of three samples.



SELENIUM ADDITIONS LOWERED MERCURY CONCENTRATIONS IN FISH AND INCREASED SELENIUM CONCENTRATIONS IN GONADS

ABSTRACT

It is uncertain whether selenium (Se) toxicity to fish can be avoided when adding enough Se to significantly lower mercury (Hg) concentrations in fish. If low Se concentrations are effective without causing toxicity, Se could be applied to lower Hg concentrations in fish from lakes and reservoirs. The mechanisms by which Se may lower Hg concentrations in fish are uncertain, but may include modifying Hg methylation and bioaccumulation and increasing loss of Hg from fish. We investigated the lowest range of Se concentrations that could lower Hg in fish. Our null hypotheses were: 1) Se additions do not affect Hg methylation and bioaccumulation; and 2) low-level Se additions do not impose toxicity to yellow perch. We added Se as sodium selenite (Na_2SeO_3) to mesocosms at the Experimental Lakes Area, Ontario, Canada, at target concentrations of 0, 0.1, 0.2, 0.4, 0.8, and 1.6 µg/L. We also added 0.3 µg/L of a stable isotope of Hg (^{198}Hg referred to as spike Hg) as mercuric chloride. To determine the impact of Se addition on Hg bioaccumulation, we measured spike Hg in periphyton, zooplankton, Chironomid larvae, and yellow perch. We also used stable nitrogen (N) isotopic signals to determine whether fish from different treatments had similar trophic positions. We measured Se concentrations in gonads of fish to reveal whether they were below the suggested toxicity threshold. Concentrations of Se in filtered water from mesocosms were close to intended treatment concentrations throughout our experiment. Spike Hg concentrations in the muscle of fish were negatively correlated with Se concentrations in filtered water ($p=0.004$; $r^2=0.88$). Bioaccumulation factors (BAFs) of spike THg in fish ($p=0.03$) and of spike methyl mercury (MeHg) in zooplankton ($p=0.03$) and Chironomid larvae ($p=0.049$) showed negative relationships with Se concentrations in filtered water. Therefore low-level additions of Se in water lowered spike Hg concentrations in muscle of fish and lowered the BAFs of Hg or MeHg in the lower food web. The proportion of spike Hg in muscle of fish that occurred as MeHg was 65%, indicating that spike Hg was methylated and bioaccumulated. In addition, fish fed at similar trophic positions among mesocosms as indicated by stable N isotopic values. Because significant relationships were observed in the food web, but not in abiotic compartments, we suspect the most important interaction between Se and Hg occurs in the food web. Concentrations of Se in gonads of fish positively correlated with Se concentrations in filtered water ($p=0.0002$; $r^2=0.97$). Concentrations of Se in gonads were higher than expected after only

eight weeks. Concentrations of Se in the gonads of yellow perch exceeded the toxicity threshold when there was 0.5 to 0.7 μg/L of Se in water. Therefore we rejected our null hypothesis that Se added to water did not directly increase concentrations of Se in gonads of yellow perch. Thus, adding Se elevated the risk of Se toxicity.

INTRODUCTION

Cycling of Hg and Se

Mercury (Hg) is a widespread neurotoxic contaminant and a major reason for fish consumption advisories in North America. In 2006, 3 851 fish advisories were issued due to Hg that restricted fishing in 38% of lake acres, 26% of river miles, and 65% of coastline in the United States (USEPA 2007). In four studies, adding selenium (Se) lowered Hg concentrations in muscle of fish (Rudd *et al.* 1980; Turner and Rudd 1983; Paulsson and Lundbergh 1989; 1991); these results were site specific, concentration dependent; and varied with food web structure. These studies used relatively high concentrations of Se and they did not assess the endpoints of Se toxicity or the processes that lowered Hg concentrations.

There are different ways by which Hg and Se could interact in freshwater environments. Selenium may redistribut or increase loss of Hg from fish (Pedersen *et al.* 1998; Bjerregaard *et al.* 1999). It may modify Hg bioaccumulation (Turner and Swick 1983; Turner and Rudd 1983), and it may lower the rate of Hg methylation in aquatic environments (Jackson 1991; Chen *et al.* 1997; Jin *et al.* 1999). The relative importance of each mechanism is unknown.

Where Se concentrations are low, it may be possible to add Se to water with the intention of lowering Hg concentrations in muscle of fish without causing Se toxicity to fish. Lowering Hg concentrations by using Se is only desirable if the Se does not cause adverse effects. Although Se is essential for production of amino acids, too much causes deformities in the offspring of fish. Fish require 0.1 to 0.5 µg/g Se in their diet (Hodson and Hilton 1983), but more than 3 µg/g in the diet can cause toxicity (reviewed by Lemly 1997b). Deformities are caused by Se substitution for sulfur (S) during protein synthesis, changing the structure of proteins, and therefore altering protein function. The accepted threshold for Se toxicity is 7.9 µg/g (5.8 µg/g before winter) in fish tissue (USEPA 2004) and in gonads specifically, the suggested threshold for Se toxicity in fish is 10 µg/g (wet weight [w.w.]; Lemly 2002a).

Objectives

We had two objectives. Our first was to determine if low-level additions of Se, at concentrations less than 2 µg/L, could cause lower Hg concentrations in muscle of fish. Our second was to assess if low-level Se additions cause Se toxicity to fish. Our first null hypothesis states that Se additions do not effect Hg concentrations in fish. Our second null hypothesis was that Se additions do not cause Se concentrations in gonads of fish to exceed the toxicity threshold.

METHODS

Location

We performed this research in 2005 at the Experimental Lakes Area (ELA), which is located on the Precambrian Shield 70 km southwest of Kenora in northern Ontario (49°40' N, 93°43' W). The forest is predominantly jack pine intermixed with white birch (Brunskill and Schindler 1971). The area is characterized by numerous small lakes on glacially scoured bedrock. Lake 239 was selected for this study because it has a protected bay with sandy sediments. Lake 239 is oligiotrophic and has a circum-neutral pH (Chapter 4; Table 1).

Materials

Mesocosms were each 10 m in diameter with a maximum depth of 2.8 m. Based on mean depth the calculated volume of the mesocosms was 196 000 L each. Styrofoam collars covered with vinyl and framed with aluminium piping supported the surface of the mesocosms and were anchored in place. Cylindrical walls were made of flexible impermeable woven plastic and were sealed to the sediment surface with sandbags. Water inside the mesocosms could not circulate with the lake and was in contact with the bottom sediment. We snorkelled and SCUBA dived, with care to not disturb sediment, in and around mesocosms to look for tears and broken seals.

Additions of Hg and Se

We increased the sensitivity of our measurements by adding a stable isotope of Hg (^{198}Hg; % isotope) as mercuric chloride (HgCl). We will refer to the stable isotope of ^{198}Hg as spike Hg. Spike Hg was added as a tracer that could be analyzed separately from ambient Hg. Therefore spike Hg was used to monitor the effects of adding Se. Even though only a small amount of the spike Hg was methylated and returned to the water, this small amount of spike methyl mercury (MeHg) was an important source of MeHg to the food web. We added 5.8 mg (0.3 μg/L) of spike Hg to each mesocosm. This amount was about ten times the mean annual atmospheric deposition at the ELA of 7 $\mu g/m^2 \cdot y$ (St. Louis *et al.* 2001). The isotopic Hg was dissolved in nitric acid (5%) and then measured into Teflon bottles. This solution was mixed with about 500 mL of water from Lake 239 the night before treatment addition.

We used a regression-based model for our study design. We added 0, 0.1, 0.2, 0.4, 0.8, and 1.6 μg/L of sodium selenite (Na_2SeO_3) to different mesocosms. Concentrations of Se were

known to be low in surrounding lakes (Mailman and Bodaly, unpublished data) and we assumed Se concentrations in water of approximately 0.1 μg/L. Treatments were randomly assigned to mesocosms. We added Se on 22 June 2005 after sunset and used and electric trolling motor to mix the treatments into the water. We added spike Hg to all mesocosms, waited an hour, and then added Se to all mesocosms.

Analysis of Se, total Hg (THg), and MeHg were conducted on muscle, liver, gills, and brains of fish to look at tissue distributions, entry routes, and dynamics of Hg and Se in tissues. Only Se species were analyzed in gonads of fish. Zooplankton, Chironomid larvae, and periphyton were analyzed for Se species and MeHg. Stable ^{13}C and ^{15}N were analyzed in the surface sediment, periphyton, zooplankton, Chironomid larvae, and fish muscle. Analysis of N for surface sediment was not completed.

Sample collection

Eight weeks after the treatment addition on 16 August, we sampled all environmental compartments. Mesocosms were decommissioned beginning on 29 August.

Periphyton

On 10 July, we suspended three strips of 5 cm wide wall material from a cedar board in each mesocosm and each strip was weighted at the bottom. On 16 August, two people wearing clean gloves removed the periphyton strips. One person cut the strip off of the board, gently rolled the strip underwater, and then cut off the weight. The second person held a large polyethylene bag open while the first person lifted the strip out of the water and into the bag. We placed periphyton strips in a cooler on ice. Wearing gloves, we scraped periphyton off of strips into polyethylene bags using our fingers. With lake water, we rinsed periphyton from the strips into respective bags. The periphyton slurry was transferred into an acid-washed glass beaker and water was added to make 200 mL. Samples of periphyton were frozen at -4°C in polyethylene bags before analyses of Se, MeHg, and stable isotopes of C and N.

Zooplankton

Zooplankton were captured when the mesocosms were deployed. In addition, zooplankton were added from the lake to supplement the population trapped during mesocosm deployment. To inoculate the mesocosms with zooplankton, a net (0.5m in diameter, 150 μm mesh) was towed behind a boat. The collected plankton were diluted in a volume of water, and

then the same amount of water and zooplantkton was distributed among mesocosms. Zooplankton samples for analyses of Se, MeHg, and stable isotopes of C and N were collected using horizontal tows of a 150 μm mesh Wisconsin net attached to a wooden pole. Care was used to collect samples with less than 10% of algae, sand, or suspended particulate matter, and the net was rinsed between sample collections. Zooplankton samples were frozen soon after collection.

Macro-Invertebrates

Macro-invertebrates were captured when mesocosms were deployed. They were collected for analyses of Se, MeHg, and stable isotopes of C and N using a ponar dredge that collected approximately 2 L samples. A series of brass sieves were used to separate invertebrates from sediment. Invertebrates were picked out from the remaining sediment and lake water and placed in plastic trays using plastic pipettes and tweezers. Benthic invertebrates were separated by class or order and frozen in polyethylene bags. In August, they were collected from inside each mesocosm after all other sampling was complete. Chironomid larvae were used to compare treatments as they were present in all mesocosms.

Fish

We stocked mesocosms with yellow perch (*Perca flavescens*) that were native to Lake 239. Before the experiment we fished the mesocosms extensively using baited minnow traps and gill nets. Yellow perch were then collected from Lake 239 using hoop and trap nets and beach seining. A sub-sample of 30 fish was randomly taken in June for time zero analyses. Fish added to mesocosms were anesthetized 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 three weeks after treatment addition to maintain 18 fish per mesocosm. The duration of exposure of an individual fish ranged from five to eight weeks.

We searched for and removed dead fish every two to four days. Final sample sizes of fish were variable due to fish mortality. Sample numbers were as follows: Treatment 0-four fish (two of these were found floating and no liver was available); Treatments 0.1 and 0.2-eight fish each; Treatment 0.4-16 fish; Treatment 0.8-four fish; and Treatment 1.6-2 fish.

Fish were collected using minnow traps baited with bread, 6, 8, and 10 mm gill nets, a beach seine net, and by snorkeling and SCUBA diving with dip nets. Fish were killed by an overdose of MS-222. We removed gonads, liver, kidney, brain, and gills, which we froze

individually in polyethylene bags. Muscle, liver, gills, and brain of yellow perch were analyzed for THg and TSe. Ten samples of each organ were analyzed for Se. Ambient and spike MeHg concentrations were analyzed in 16 samples of muscle from three mesocosms. Only Se was analyzed in gonads of fish.

Sub-samples of skinless dorsal muscle were taken from frozen yellow perch. We calculated the bioaccumulation factor (BAF) in periphyton, zooplankton, Chironomid larvae, and yellow perch tissues. BAFs were calculated as the dry weight concentration (ng/g) of MeHg in biota divided by the concentration of MeHg in filtered water (ng/mL). The BAF gives a measure of the process of MeHg transfer from the water to the biota and therefore may help to explain the mechanism or efficiency of MeHg bioaccumulation.

Analyses

All organs and muscle were weighed before and after freeze-drying to calculate a ratio of wet to dry weight. After freeze-drying sediment, periphyton, zooplankton, invertebrates, and tissues of fish, we weighed sub-samples on a Perkin Elmer microbalance (2 to 10 mg for Hg analyses and 10 to 20 mg for Se analyses). We used an acid-rinsed stainless steel scoop to transfer the material into trace-metal-clean weigh boats. Sub-samples were placed in Teflon vials that had been washed in hot nitric acid. Vials were stored in sealed polyethylene bags.

Analyses of Hg

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) in 20 mL borosilicate vials at 120°C for four hours. Digesting samples were heated in an aluminium hot block. Tissues were diluted with 20 mL of Milli-Q water after digestion. Mercury-II in digestate was reduced to Hg^0 using 3% (w/v) $SnCl^2$. Gaseous elemental Hg^0 was separated from solution by a cold vapour generation system. Gaseous Hg^0 was purged with mercury-free argon gas and detected on ICP-MS (Hintelmann and Ogrinc 2003).

For MeHg analysis, 1 ng/mL of enriched $Me^{201}Hg$ and 5 mL of KOH-methanol was added to 2 to 10 mg of tissue. The samples were covered and heated to 50°C for 24 hours in a water bath and then diluted to 20 mL with deionized water. Digests were stored at 4°C until analyses when it was ethylated using sodium tetraethyl borate. Ethyl Hg was purged from the distillate and collected on Tenax traps. Heating these traps released the Hg which was separated by gas chromatography.

Tubes of digestion blanks were treated the same as those for tissues. Certified reference material (CRM) was prepared and digested in the same manner as samples of tissue. DORM-2 (National Research Council of Canada) was analyzed for THg in tissues and oyster tissue was analyzed for MeHg in tissues. The measured and certified values were not statistically different. The limit of detection (LOD) for ambient MeHg in zooplankton and Chironomid larvae was 0.02 to 1.0 ng/g (dry weight [d.w.]) and in fish was 1.0 ng/g (d.w.) for THg and 2.0 ng/g (d.w) for MeHg. For spike Hg, the LOD was 0.5% of the ambient Hg concentration in each sample.

Analyses of Se

Selenium was detected by atomic fluorescence hydride generation spectroscopy using a PS Analytical Millennium Excalibur Model 10.005 at Flett Research, Ltd., Winnipeg, Manitoba. To measure TSe, 200 µL of BrCl was added to 9.35 mL of each sample of filtered water. The solution was heated at 93 to 97°C for 15 minutes and then cooled. It was adjusted to 4 N 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. They were then heated to 150°C for six hours, cooled, and then made up to 10 mL with deionized water. Then 1 mL of digest solution plus 8.35 mL of deionized water was heated to 93 to 97°C for 15 minutes, cooled, adjusted to 4 N HCl, and Se^{4+} was analyzed. For Se^{4+} plus Se^{6+}, the above process was followed by reheating in the same manner, cooling, and then analyzing. 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 to 97°C for 15 minutes, cooled, adjusted to 4 N HCl, reheated in the same manner, cooled, and 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 (standard deviation). We measured 3.24 +/- 0.12 µg/L (90% recovery) and 3.76 +/- 0.37 µg/L (104.4 % recovery).

Tubes for analytical blanks were treated the same as those for samples of tissue. Measurements of Se concentrations in samples were repeatable. Triplicate analysis of water had a mean recovery of 106.3%. The certified reference material for invertebrates, muscle, gill, and gonads was DORM-2. Its certified valued is 1.4 +/- 0.09 mg/kg. We measured 1.35 mg/kg +/- 0.09 in zooplankton (96.4% recovery), 1.29 mg/kg +/- 0.14 in Chironomid larvae (92.1%

recovery), 1.45 mg/kg +/- 0.05 in gills (103.6% recovery), and 1.30 mg/kg +/- 0.06 in gonads (92.9% recovery). The 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). For brain the CRM was LUTS-1 certified at 0.641 mg/kg +/- 0.054 and measured 0.66 mg/kg +/- 0.07 (103% recovery).

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

The source of carbon (C) at the base of food webs and the relative trophic positions in a food chain can be determined by small changes in stable isotope ratios. Relative trophic levels of organisms can be identified by $\delta^{15}N$ values (Cabana and Rasmussen 1994). The length of a food web affects Hg concentrations in fish as biomagnification of contaminants is greatest in lakes with a longer food web. Concentrations of Hg are significantly predicted by trophic position (Cabana and Rasmussen 1994; Kidd *et al.* 1995). Therefore the importance of assessing the C source and trophic level of fish is to determine whether differences among mesocosms are due to treatments or other factors.

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 sub-samples of 1 mg +/- 0.2 mg were combusted to convert organic C to CO_2 and N to N_2. All samples are reported against Vienna Pee Dee Belemnite (VPDB) 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

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 running statistical analysis using Statistical Analysis Software (SAS) version 9.1.

For yellow perch, we modeled the spike THg concentrations as a function of Se 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 ANOVA with tukey multiple comparisons for

data sets with equal sample sizes and the 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.

RESULTS

For each environmental compartment, we report whether spike Hg was detected, the range of ambient and spike Hg concentrations, the relationships between ambient or spike Hg concentrations in compartments versus concentrations of Se in filtered water, and whether these relationships were significant. Where applicable, we report the percentages of THg that occurred as MeHg for both spike and ambient Hg. Data for all compartments are from the final sample date unless otherwise specified.

Did Se concentrations in filtered water correspond with target Se concentrations?

Selenium was detected in every mesocosm on every sample date (Chapter 4). Measured concentrations of total Se (TSe) in filtered water were similar to target concentrations in both July and August (Figure 1). In July, Treatment 0 contained about 0.1 µg/L of Se and Treatment 0.1 and 0.2 contained about 0.2 µg/L of Se because of the presence of ambient Se. All other treatments had Se concentrations that were close to the concentrations of Se that were intended. In August, Treatments 0, 0.1, 0.2, and 0.4 had Se concentrations in filtered water that measured 0.3, 0.3, 0.8, and 0.7 µg/L, respectively. The sample from Treatment 0.8 in August was lost. In August, Treatment 1.6 contained about 1.2 µg/L of Se. In Treatments 0 to 0.4, concentrations of Se increased from July to August (perhaps due to evaporation of water from the mesocosms), contrary to our expectation that concentrations of Se in water would decrease due to sedimentation or evasion. Concentrations of Se in filtered water in Treatment 1.6 decreased from July to August from about 1.5 to 1.2 µg/L. There was a positive relationship between mean Se concentrations that were measured and intended Se treatments (r^2=0.98, $p<0.0001$; Chapter 4). Water chemistry variables were similar among mesocosms. Percent carbon in sediment was also similar among mesocosms, but was higher in the mesocosm (Treatment 0.4) that was nearest to the inflow. In addition, concentrations of chlorophyll a and dissolved organic carbon were also high in Treatment 0.4 and Treatment 0.2. The effect of higher carbon is likely important because this mesocosm had the most turbid water, coldest temperatures, and greatest survival of fish.

Did Se addition affect Hg bioaccumulation?

At the end of our experiment, spike Hg was detected in the muscle of all yellow perch from our mesocosms (Figure 2). 95 to 97% of the THg in the muscle of yellow perch was present as ambient THg. Concentrations of ambient THg in muscle ranged from 500 to 800 ng/g (d.w.). The range of spike THg concentrations in muscle was from 15 to 40 ng/g (d.w.). Concentrations of spike MeHg in muscle were five to 20 times greater than those in zooplankton and benthic invertebrates. 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), but concentrations of spike THg in muscle correlated negatively with Se concentrations in filtered water (p=0.0036, r^2=0.90, $\log(Hg_{Muscle})=1.24-0.48*\log(TSe_{Water})$).

In liver of yellow perch, concentrations of ambient THg were slightly higher than spike THg concentrations (Figure 3). 42% of the THg in liver of yellow perch was present as ambient THg. Concentrations of spike THg were 10 to 20 times higher in liver than those in muscle. This may indicate the spike Hg was first going to the liver after assimilation. Ambient THg concentrations in liver of yellow perch ranged from 250 to 700 ng/g (d.w.) and were related to TSe concentrations in filtered water (r^2=0.80, p=0.02). Spike THg concentrations in liver ranged from 230 to 800 ng/g (d.w.) and also were related to TSe concentrations in filtered water using a quadratic model (r^2=0.97, p=0.007).

70 to 80% of the THg in the gills of yellow perch was present as ambient THg (Figure 4). Ambient THg concentrations in gills ranged from 225 to 325 ng/g (d.w.) and were not related to Se concentrations in filtered water (r^2=0.20, p=0.38). Spike THg concentrations in gills ranged from 50 to 130 ng/g (d.w.) and were not related to Se concentrations in filtered water (r^2=0.59, p=0.07).

55 to 75% of the THg in the brain of yellow perch was present as ambient THg (Figure 5). These concentrations ranged from 205 to 325 ng/g (d.w.). Spike THg concentrations in brain ranged from 52 to 135 ng/g (d.w.). Ambient THg concentrations in brain were not related to Se concentrations in filtered water (r^2=0.18, p=0.41), nor were those of spike THg (r^2=0.22, p=0.34).

We detected spike MeHg concentrations in zooplankton from all mesocosms on all sample dates (Figure 6). Ambient MeHg concentrations in zooplankton ranged from 45 to 160 ng/g after eight weeks and were not related to TSe concentrations in filtered water (r^2=0.53, p=0.10). Eight weeks after treatment addition, spike MeHg concentrations ranged from about 5 to 22 ng/g (d.w.) in zooplankton. At this time, spike MeHg concentrations in zooplankton did

not exhibit a statistically significant decreasing relationship with TSe concentrations in filtered water (r^2=0.62, p=0.06).

We detected spike MeHg in Chironomid larvae from all mesocosms eight weeks after treatment additions (Figure 7). Ambient MeHg concentrations in Chironomid larvae were highest in Treatment 0.4, but were lower at higher Se concentrations. Concentrations of ambient MeHg in Chironomid larvae ranged from 30 to 60 ng/g and were not related to TSe concentrations in filtered water (r^2=0.03, p=0.75). In Chironomid larvae, concentrations of spike MeHg were similar among treatments. Concentrations of spike Hg in Chironomid larvae ranged from about 1 to 5 ng/g (d.w.). In addition, spike MeHg concentrations in Chironomid larvae were less than those in zooplankton and greater than those in periphyton. There was no significant relationship between spike MeHg concentrations in Chironomid larvae and concentrations of TSe in filtered water (r^2=0.29, p=0.27).

Spike MeHg was detected in periphyton after six weeks of exposure. Ambient MeHg concentrations in periphyton ranged from 4 to 10 ng/g (d.w.; Figure 8) and were not related to TSe concentrations in filtered water (r^2=0.006, p=0.89). In all treatments, spike MeHg concentrations in periphyton were near 1 ng/g (d.w.), which were greater than those in surface sediment (Chapter 4). In periphyton, spike MeHg concentrations also were unrelated to TSe concentrations in filtered water (r^2=0.07, p=0.62).

Spike MeHg was incorporated into all compartments of the food web including muscle of yellow perch. There was more ambient MeHg than spike MeHg in zooplankton, Chironomid larvae, and periphyton (Figure 9). In zooplankton, Chironomid larvae, and periphyton, the spike MeHg occurred as 10 to 20, 3 to 10, and 9 to 19% of the ambient MeHg, respectively. Ambient MeHg concentrations in muscle of yellow perch ranged from 600 to 850 ng/g (d.w.; Figure 10). The concentration of spike MeHg in muscle was 50 ng/g (d.w.). The amount of ambient THg in muscle that occurred as MeHg was slightly less than 100% (Figure 11), whereas about 65% of the total spike Hg in muscle was present as spike MeHg.

We evaluated whether Se affected bioaccumulation processes. We analyzed, using multiple regressions, the relationships between ($\log_{10}$) bioaccumulation factors (BAFs) of Hg or MeHg as a function TSe and Hg concentrations in filtered water. These analyses were done for each of zooplankton, Chironomid larvae, periphyton (Figure 12), and tissues of yellow perch (Figures 13). The 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. Zooplankton had higher BAFs of MeHg than Chironomid larvae and periphyton. The BAFs of spike THg in muscle of yellow perch ranged from 3.2 to 3.7,

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, respectively. The BAFs of spike THg for the various tissues of fish were less than those in the lower food web. Relationships of BAFs of spike MeHg in zooplankton ($p=0.03$; $r^2=0.75$; $\log(BAF_{MeHg})=2.696-0.909*\log(TSe_{Water})$ and Chironomid larvae ($p=0.049$; $r^2=0.66$; $\log(BAF_{MeHg})=2.28-0.596*\log(TSe_{Water})$ with TSe concentrations in filtered water were significant, while in periphyton the relationship was not significant ($p=0.36$; $r^2=0.21$). The relationship between the BAF of spike THg in muscle of yellow perch as a function of TSe concentrations in filtered water was significant ($p=0.03$; $r^2=0.73$; $\log(BAF_{THg})=\log(3.3)-0.43*\log(TSe_{Water})$, but in other tissues it was not (liver: $p= 0.17$; $r^2=0.41$, gills: $p=0.10$; $r^2=0.54$, brain: $p=0.56$; $r^2=0.09$). Usually Hg is bioaccumulated as MeHg.

Did fish eat at the same trophic level among mesocosms?

To rule out the possibility that differences in concentrations of Hg in fish among mesocosms were due to differences in food web structure, we evaluated the ^{15}N and ^{13}C signatures in muscle of fish. At the beginning of the experiment, muscle of yellow perch contained 5.9 +/-0.6 $^{o}/_{oo}$ $\delta^{15}N$ (Figure 14). After eight weeks in the mesocosms, mean $\delta^{15}N$ values in muscle ranged from 5.6 +/-0.3 to 5.9 +/-0.2 $^{o}/_{oo}$ (Figure 15). Final trophic positions of yellow perch as indicated by $\delta^{15}N$ in muscle were not significantly different among mesocosms ($p=0.69$; Table 2). Therefore we failed to reject our null hypothesis that there were no differences in trophic position of these fish among treatments.

At the start of the experiment, muscle of yellow perch had $\delta^{13}C$ values of -25.8 $^{o}/_{oo}$ +/-1.7 (Figure 14). Values of $\delta^{13}C$ for muscle of yellow perch after the experiment ranged from -26.9 +/-1.3 to -23.3 +/-1.2 $^{o}/_{oo}$ (Figure 15). The C/N ratio of muscle ranged from 4.1+/-0.3 to 4.7+/-0.5, indicating the fatty acid content of muscle was fairly consistent in fish among mesocosms. Muscle of yellow perch was significantly more depleted in ^{13}C in mesocosm 6 than in all other mesocosms at $p<0.0001$ (Table 1). Although this is significantly different, the magnitude is small. The source of C to yellow perch in mesocosm 6 may have differed from the other mesocosms. However, the $\delta^{13}C$ signature in muscle before and after the experiment was similar to that of Chironomid larvae. Therefore we surmise that the source of C to these fish before and after the experiment was probably similar.

Did Se concentrations in water affect Se concentrations in gonads?

In all yellow perch that had developed gonads, Se was measured after a maximum exposure of eight weeks (Figure 16) and ranged from 3 to 12 μg/g (d.w.). Concentrations of TSe in gonads of yellow perch significantly correlated with TSe concentrations in filtered water (r^2=0.97, p=0.0002; $\log(TSe_{Gonads})=0.995+0.673+\log(TSe_{Water})$). We rejected our null hypothesis that adding Se to water did not affect TSe concentrations in gonads. In addition, concentrations of TSe in gonads of yellow perch positively correlated with those in muscle (r^2=0.67, p<0.0001; $\log(TSe_{Gonads})=0.352+0.772*\log(TSe_{Muscle})$; Figure 17). The proportion of yellow perch that developed gonads was 98%. The mean concentration of Se in gonads in Treatment 0.8 was 8.9 +/- 2.2 μg/g (maximum of range in individual fish: 11.7 μg/g) and in Treatment 1.6 the mean concentration was 13.1 +/- 5.6 μg/g (maximum of range in individual fish: 17.0 μg/g). Thus the highest Se treatment had a mean concentration in gonads greater than 10 μg/g. In addition, the two highest treatments contained fish whose gonads had Se concentrations greater than 10 μg/g. The most widely accepted toxicity threshold of 7.9 μg/g in tissues of fish was met at 0.7 μg/L of Se in water and the more conservative threshold of 5.9 μg/g (before winter) was met at 0.5 μg/L of Se in water.

DISCUSSION

Adding Se lowered Hg concentrations in muscle and liver of yellow perch

We rejected our null hypothesis that Se did not affect Hg concentrations in muscle and liver of yellow perch. In muscle and liver, total Hg concentrations were lower in treatments with higher Se concentrations. No significant differences in muscle of yellow perch $\delta^{15}N$ values were observed among mesocosms, indicating that these fish were generally feeding at the same trophic level. At 1.3 μg/L of Se, mean spike Hg concentrations in muscle of yellow perch were 59% lower than in control mesocosms eight weeks after Se additions. Additions of Se lowered Hg concentrations in muscle of fish by the following amounts relative to the control, as calculated from our regression equation: 54% lower at 1 μg/L, 49% lower at 0.8 μg/L, 41% lower at 0.4 μg/L, and 28% lower at 0.2 μg/L. However, these mesocosms were not at steady state and these fish were exposed to Se for only eight weeks. In addition, 0.5 μg/L of Se could lower Hg concentrations by only a third in these tissues.

Our results of lowered Hg concentrations in muscle of yellow perch due to the presence of Se in water agree with other studies. However, our study used Se concentrations that were lower than in other studies. Rudd et al. (1980) saw a 50% decrease in Hg burdens in muscle of

pearl dace (*Semotilus margarita*) at 100 µg/L of Se. After six weeks, yellow perch had 20% less Hg body burden in 1 µg/L of Se and 30% less at 10 µg/L of Se; Se did not lower Hg in clams, snails, or zooplankton (Turner and Rudd 1983). Northern pike (*Esox lucius*) contained 30 to 60% less Hg after two weeks in water with 10 µg/L of Se and Se spiked food (Turner and Swick 1983). Concentrations of Hg in muscle of yellow perch dropped by 84% after three years in water with 5 µg/L of Se and lime treatment (Paulsson and Lundbergh 1989). Paulsson and Lundbergh (1991) observed 60% lower Hg concentrations in muscle of yellow perch at 1 to 2 µg/L of Se and lime treatment, and these were 85% lower at 3 to 5 µg/L of Se and lime treatment after two years. Our study and the literature indicate that Se additions do lower Hg concentrations in muscle of fish.

Reductions in Hg concentrations in muscle of fish with Se additions could arise from several different mechanisms. For example, Se additions could affect Hg methylation and/or bioaccumulation. Although relationships between MeHg concentrations in zooplankton and Se concentrations in filtered water were not statistically significant, the concentrations of Hg were lower at higher Se concentrations. Zooplankton acquire MeHg from water or from ingesting particulate matter (Monson and Brezonik 1998; Peech Cherewyk 2002; Mason *et al.* 1996; Plourde *et al.* 1997). We could not detect an effect on MeHg concentrations in filtered water or particulate matter due to Se concentrations in filtered water (Chapter 4). However, BAFs of spike MeHg in zooplankton and Chironomid larvae were significantly lower at higher concentrations of TSe in filtered water. This could indicate that Se was affecting MeHg trophic transfer in the lower food web.

Results of this study suggest that the mechanisms by which Se additions lowered Hg concentrations in the food web were likely modifications of Hg uptake or retention and not due to lowered Hg methylation. Significant relationships between Hg concentrations in muscle of yellow perch and Se concentrations in filtered water also suggest the mode of action was a modification of Hg bioaccumulation. Because there were no significant relationships between THg or MeHg concentrations in filtered water, particulate matter, and surface sediment with TSe concentrations in filtered water (Chapter 4), we assume that interactions between Hg and Se related to methylation were not of great importance during eight-weeks of exposure.

Spike Hg methylation and bioaccumulation

Very little of the spike Hg was methylated and returned to the water column (Chapter 4). Most of the MeHg in filtered water was ambient MeHg whereas most of the THg was spike

THg. To summarize our results, we rejected our null hypothesis that Se added to water did not affect Hg concentrations in muscle and liver of fish. We also found significant relationships for BAFs of Hg in muscle of fish, zooplankton, and Chironomid larvae compared with Se concentrations in filtered water. We failed to reject our null hypothesis that added Se affected spike MeHg or THg concentrations for gills, brain, zooplankton, Chironomid larvae, and periphyton, but concentrations were often in the hypothesized direction.

Spike Hg was present in all yellow perch, but not at steady state. Another mesocosm study found about half of the yellow perch contained spike THg after five weeks and almost all of the yellow perch contained spike THg after 10 weeks (Orihel 2005). A different mesocosm study found that 15% of the newly acquired Hg was spike Hg after one year (Paterson *et al.* 2006). Paterson *et al.* (2006) also observed that steady state with regard to spike Hg was not achieved in fish even after two summers of exposure.

In muscle of yellow perch, about 65% of the spike THg was present as spike MeHg without achieving steady state conditions. This is evidence for spike MeHg bioaccumulation by yellow perch. In a mesocosm study that added radioactive Hg to water and sampled one-year-old yellow perch after three weeks, 85 to 100% of radioactive Hg assimilated into muscle of fish was MeHg (Hecky *et al.* 1991). Also, in age one yellow perch from Lake 658 at the ELA that were exposed to spike Hg for one year, the entire spike THg burden was present as spike MeHg (Van Walleghem *et al.* 2007). Our study and the literature indicate that spike Hg is methylated, taken up by the lower food web and then incorporated into tissues of fish. Also, there was much more spike THg in liver than in gills, which suggests that most MeHg uptake by fish is via the diet, not through the gills from water, in agreement with other studies.

There was much more spike THg in liver than in muscle. It appears from our study that spike THg first enters the liver and is then transferred to muscle. In past studies, Hg has been shown to be adsorbed by the intestine, transferred through blood, taken up first by liver and other organs, and then redistributed to muscle tissue (reviewed by Wiener *et al.* 2003). An oral dose of MeHg to sheepshead minnows was found in liver after 1.5 days and was transferred to the rest of the body after approximately 35 days (Leaner and Mason 2004). In a study of the dynamics of spike Hg in yellow perch, Van Walleghem *et al.* (2007) found that Hg was transferred from liver to muscle within 90 days (estimated 24 day half-life of THg in liver). In our study, it appeared that Se decreased the amount of spike THg accumulated in liver and thus decreased the concentrations of spike THg in muscle of yellow perch. This implies that

modification of Hg uptake from food is the most likely pathway by which Se modified Hg cycling.

Se concentrations increased in gonads of fish

We rejected the hypothesis that adding Se at low-levels did not elevate Se concentrations in gonads. Rapid bioaccumulation of Se by yellow perch resulted in quickly elevated Se concentrations in gonads of fish in only eight weeks. Presumably, fish exposed to these concentrations for longer would accumulate higher Se concentrations in gonads. These concentrations of Se in gonads do not represent steady-state concentrations. The main concern for Se toxicity is during development of fish larvae (Lemly 1998). Increases in Se concentrations in gonads increase the risk of Se toxicity. Fish larvae acquire Se from the yolk sack, where Se is transferred from the liver to their gonads during egg development. The suggested Se toxicity threshold is 7.9 µg/g (d.w.) in fish tissue (USEPA 2004) and 10 µg/g (d.w.) in gonads of fish (Lemly 1998). Using our regression equation we estimated that this threshold was crossed in our study at Se concentrations in filtered water that ranged between 0.8 and 1.0 µg/L. Turnover rates of Se in gonads were not found in the literature. However, about 1 ng/L of ^{75}Se was added to a lake after which fathead minnows steadily accumulated a maximum of 30 cpm/g in their gonads at 250 days after addition, and then this decreased to 15 cpm/g at 350 days after addition (Harrison *et al.* 1990).

The positive relationship between concentrations of TSe in muscle and those in gonads can be used to estimate Se concentrations in gonads when Se concentrations in muscle are known. As a result, non-lethal sampling of muscle of fish can be employed (Baker *et al.* 2004), but only when concentrations of Se in muscle are within the range reported in this regression analysis. Also, our results were not at steady state. In addition, concentrations in muscle and gonads of fish would change seasonally as a result of Se depuration from spawning. Therefore these results should be treated with caution. Similar relationships were found for other fish species. There was a strong correlation between Se concentrations in muscle and eggs of razorback suckers (Hamilton and Waddell 1994), rainbow trout, and brook trout (Holm *et al.* 2005). The correlation between Se concentrations in muscle and eggs is species specific (Palace *et al.* 2004). Limitations of using muscle to estimate the potential for selenosis in offspring of fish are small tissue mass, effects from other elements, and short biological half-lives in tissue.

Evaluation of Se addition as a strategy to lower Hg concentrations in fish

There are advantages and disadvantages of using Se to lower Hg concentraitions in fish. Se additions are effective at lowering MeHg concentrations in fish. Lowering Hg concentrations in water may protect fish embryos from Hg toxicity because waterborne Hg is known to disrupt eggs of fish and development of larvae (Hammerschmidt *et al.* 1999; Johnston *et al.* 2001; Hammerschmidt *et al.* 2002; Hammerschmidt and Sandheinrich 2005). In addition, Se could be applied selectively to treat hydroelectric reservoirs and point sources of contamination. The cost of Se addition is relatively low, and application via float plane, boat, or in suspended biodegradable rubber would be relatively inexpensive. It would be informative to learn how added Se moves through a whole system. It could provide information for Environmental Impact Assessments and cost-benefit analysis. If Hg can be lowered in lakes and reservoirs, then associated environmental impacts may be lowered. Enabling fishing for sustenance and commercial sale by lowering concentrations of MeHg in fish would give fishers more choice and empowerment.

First Nations people who live near dams may limit their fish consumption to lower their exposure to Hg. This can violate treaty rights, change social and cultural activities, and undermine fishing for sustenance and commercial sale, and therefore undermine sources of income, self-determination, and esteem. Opening the possibility of consuming piscivorous fish when necessary or desired without the high risk of Hg intoxication could help to ensure food security. Lower Hg in piscivorous fish also permits fishing for commercial sale. The commercial sale of fish would provide some income, preserve fishing as a cultural and social activity, and increase the capacity for self-determination and self-esteem of fishers.

There are also many disadvantages to using Se to lower Hg concentrations in fish. Repeated additions may be required. Se additions would likely affect downstream environments and these effects would need to be monitored closely. Selenium can accumulate in downstream riparian wetlands, which are sensitive nursery habitats for many organisms (Lemly 2002b). There is a risk of Se toxicity because the difference between essential levels of Se and levels that may cause toxicity is small (Lemly 1998). Some Se is essential for protein synthesis. Fish require 0.1 to 0.5 μg/g (d.w.) in their diet (Hodson and Hilton 1983). Toxicity has been reported when dietary concentrations are greater than 3 μg/g (Lemly 2002a). In Chapter 3, emerging insects contained 0.01 to 4.4 μg/g (d.w.) and Chironomid larvae contained 0.5 to 1.5 μg/g (d.w.) and in all of the sites except the ELARP reservoir, the lower food web met or exceeded the dietary requirements of Se in fish diet, and in some cases exceeded the toxicity threshold. There

were sufficient Se concentrations in invertebrates to satisfy Se requirements of fish in regions with low Se concentrations in water. In addition, seasonal variations of Se concentrations in water (Chapter 3) could over- or under-shoot target concentrations and either fail to cause an effect or elevate the risk of a toxic effect from Se. As well, winter stress syndrome increases Se concentrations while Se burdens remain similar in fish during the winter (Lemly 1993a; Lemly 1996) causing higher Se concentrations when toxicity is most critical, that is before or during vitellogenesis when Se is transferred to eggs. As a result, more Se could be transferred to eggs via vitellogenin. The threshold for Se in muscle of fish is 7.91 μg/g (d.w.), but if species are to over winter, they should not contain more than 5.85 μg/g (d.w.) before winter. The manpower and cost of monitoring Se concentrations throughout the target and downstream systems could be substantial. Adding one substance that could cause negative impacts to treat another could set and important precedent. The mechanisms by which Se lowers Hg concentrations in fish are poorly understood (Chapter 2). Even in systems of low Se concentrations in water, benthic invertebrates and zooplankton already contain Se concentrations that satisfy the requirement of Se as an essential element in the diet of fish (Chapter 3).

In summary, Hg concentrations in the muscle of fish and the BAFs of spike THg in muscle as well as those of spike MeHg in zooplankton and Chironomid larvae were lowered by adding Se at concentrations of only 2 to sixteen times natural concentrations. Concentrations of Se of 1 μg/L caused an estimated 54% decrease in spike THg concentrations in muscle. Fish had lower Hg concentrations, likely due to Se modifying Hg uptake in the lower food web or to lower Hg assimilation by fish. Sixty-five percent of the spike THg in muscle was present as spike MeHg. Concentration of TSe in gonads and those in filtered water correlated significantly. At 1 μg/L of Se in water the gonads contained 10 μg/g of Se, the most widely accepted toxicity threshold (Lemly 2002a). In addition, TSe concentrations in muscle significantly correlated with those in gonads, which would allow non-lethal assessment of a risk of toxicity. Concerns about elevated risk of Se toxicity lead us to conclude that addition of Se to reservoirs is not a viable technique to lower Hg concentrations in muscle of fish.

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Figure 13. BAFs of spike THg in muscle, liver, gills, and brain of yellow perch as a function of TSe concentrations in filtered water. Each data point represents the mean with one standard deviation.

Figure 14. Pre-treatment (June) data of $\delta^{15}N$ and $\delta^{13}C$ in muscle of yellow perch, Chironomid larvae, zooplankton, periphyton, and surface sediment from each mesocosm. No periphyton samples were collected in June. Yellow perch in June are from the lake that was the source of the fish. Error bars are one standard deviation (periphyton n=3; sediment n=3; yellow perch: n=20). Zooplankton samples for Se Treatment 1.6 µg/L in June were not available.

Figure 15. $\delta^{15}N$ and $\delta^{13}C$ values in muscle of yellow perch, Chironomid larvae, zooplankton, periphyton, and surface sediment from each mesocosm in August. Data points represent one composite sample of zooplankton and Chironomid larvae, periphyton (n=3); surface sediment (n=3); muscle of yellow perch (n=8, 2, 5, 4, 9, 16, respectively by mesocosm) with one standard deviation. Solid circles represent muscle of yellow perch; open circles represent Chironomid larvae; solid triangles represent zooplankton; open triangles represent periphyton; solid squares represent surface sediment (0-2 cm).

Figure 16. 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 with one standard deviation. The toxicity threshold for Se in tissue of fish is 7.91 µg/g, or 5.85 µg/g before winter. The sample size is noted above the error bars.

Figure 17. Concentrations of TSe in muscle versus concentrations of TSe in gonads of yellow perch. Each data point represents one fish. All fish were collected from mesocosms at the end of our study.

TABLES

Table 1. Summary of $\delta^{13}C$ and $\delta^{15}N$ data and significant differences calculated by ANOVA, and Tukey multiple comparisons, among mesocosms in yellow perch, Chironomid larvae, zooplankton, periphyton, and sediment (periphyton n=3; surface sediment n=3; muscle of yellow perch: n=8, 2, 5, 4, 9, 16, respectively by mesocosm). Blank cells indicate that only one sample was collected and therefore variance estimates were not available and data was not amenable to statistical analysis. Significant differences are denoted by letters. There were no significant differences among mesocosms for $\delta^{15}N$ in yellow perch muscle.

		$\delta^{13}C$			$\delta^{15}N$		
	Mesocosm	Mean	Variance	Significant Differences	Mean	Variance	Significant Differences
Yellow Perch	1	-23.70	1.65	A	5.5625	0.27	
	2	-23.34	1.20	A	5.565	0.59	
	3	-23.95	0.84	A	5.696	0.27	
	4	-24.47	1.44	A	5.925	0.24	
	5	-24.77	1.45	A	5.7838	0.44	
	6	-26.88	1.35	B	5.7231	0.43	
Chironomid Larvae	1	-23.53			2.67		
	2	-23.44			2.46		
	3	-22.85			2.66		
	4	-23.81			2.82		
	5	-37.96			-0.87		
	6	-27.8			3.46		
Zooplankton	1	-25.91			1.77		
	2	-24.43			1.73		
	3	-25.92			2.14		
	4	-26.25			1.74		
	5	-27.61			2.48		
	6	-29.09			2		
Periphyton	1	-21.92	0.4	A B	-3.935	0.39	A
	2	-21.68	0.16	A	-3.27	0.20	A B
	3	-23.76	0.29	B C	-2.71	0.64	A B
	4	-22.70	0.41	A B	-2.53	1.03	A B
	5	-25.24	0.25	C	-1.79	0.53	B
	6	-29.54	0.10	D	-1.3	0.76	B
Sediment	1	-24.34	0.63	A			
	2	-22.28	2.01	A			
	3	-24.91	0.34	A B			
	4	-24.21	1.77	A			
	5	-24.97	0.27	A B	0.97		
	6	-26.68	0.24	B	0.24	1.499066	

FIGURES

Figure 1. Concentrations of TSe in filtered water versus target TSe concentrations in water. Each bar represents one sample. Vertical bars in black, dark grey, medium grey, and light grey represent water samples from 21 June (pre-treatment), 19 July, 16 August, and the mean of 19 July and 16 August, respectively.

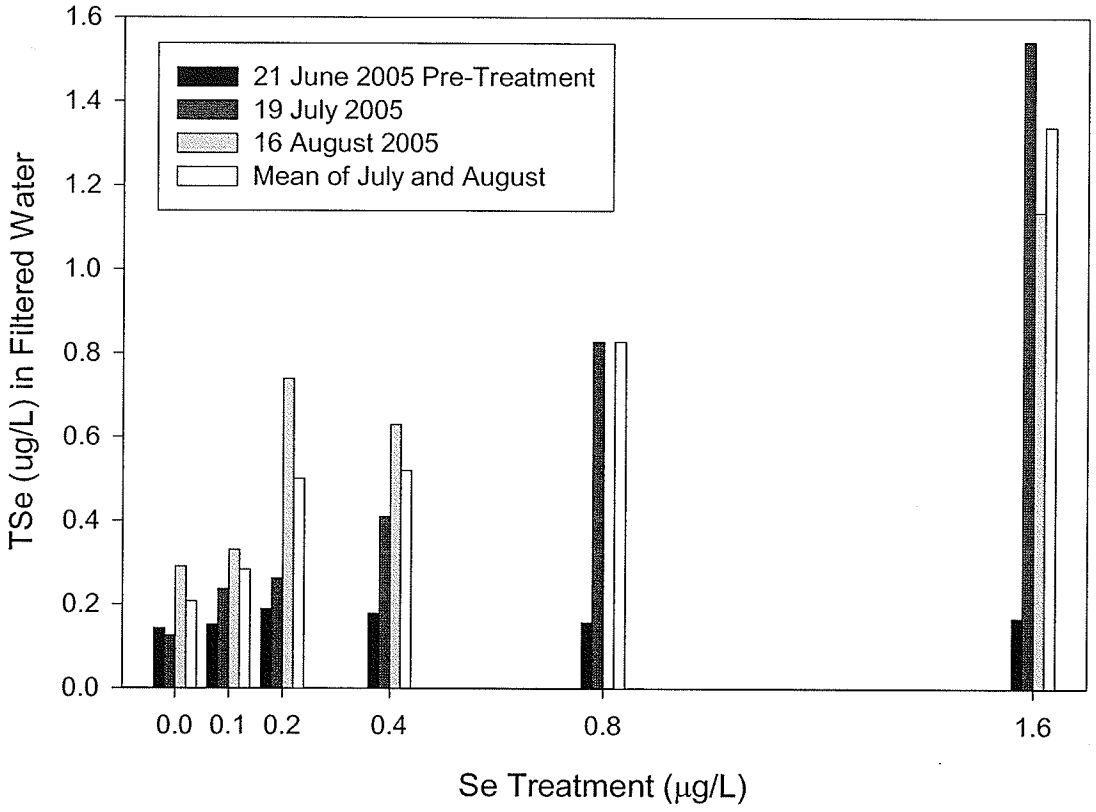


Figure 2. Ambient and spike THg concentrations in muscle of yellow perch versus Se Treatment. The ambient THg axis is on the left and the spike THg axis is on the right. The error bars are one standard deviation.

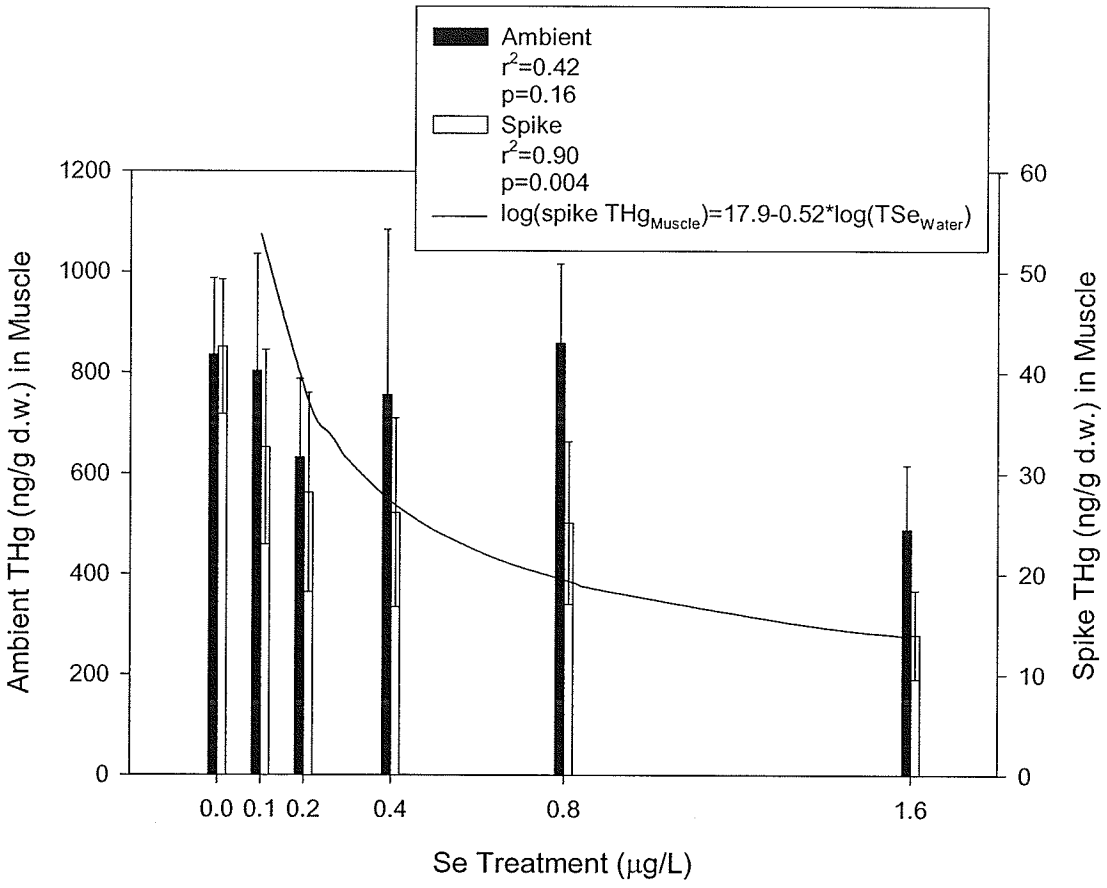


Figure 3. Ambient and spike THg concentrations in liver of yellow perch versus Se Treatment. The error bars are one standard deviation. The regression line was calculated using actual concentrations that fall within the boundaries of the regression line. The bars were graphed at intended Se concentrations for ease of visibility.

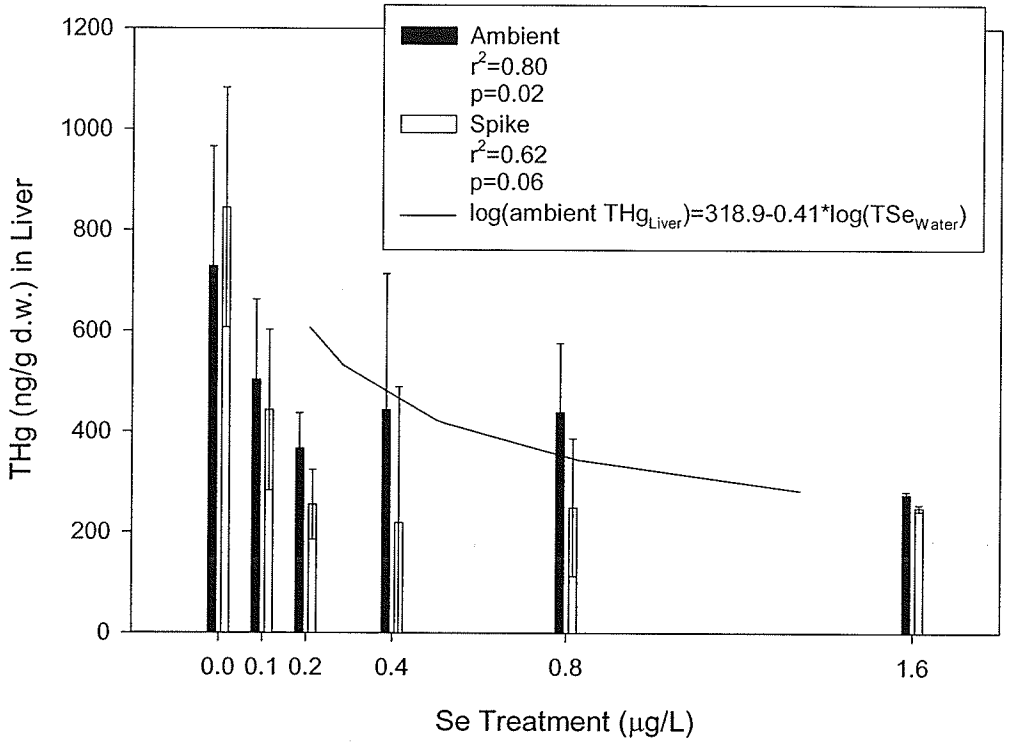


Figure 4. Ambient and spike THg concentrations in gills of yellow perch versus Se treatment. The error bars are one standard deviation.

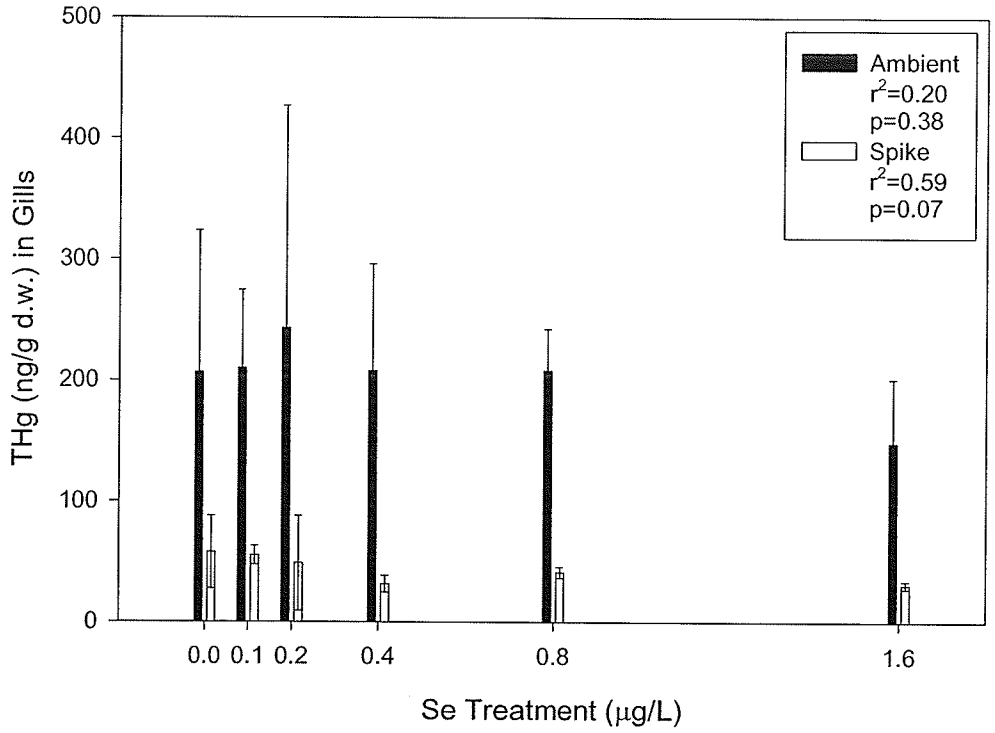


Figure 5. Ambient and spike THg concentrations in brain of yellow perch versus Se treatment. The error bars are one standard deviation.

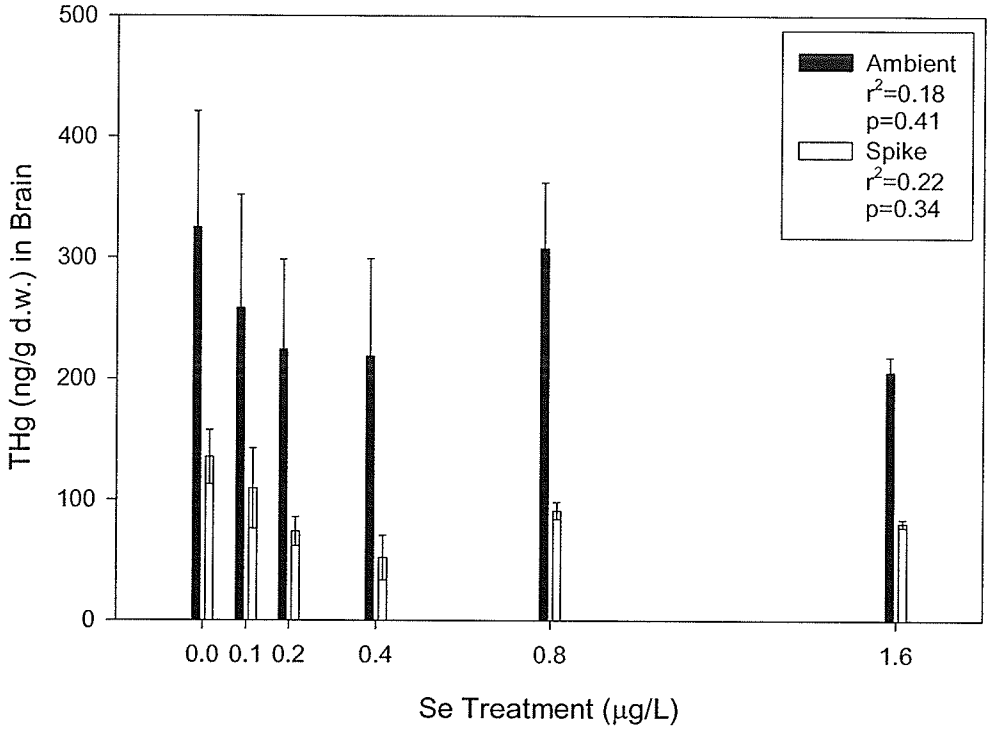


Figure 6. Ambient and spike MeHg concentrations in zooplankton versus Se Treatment in August. Each bar represents one sample.

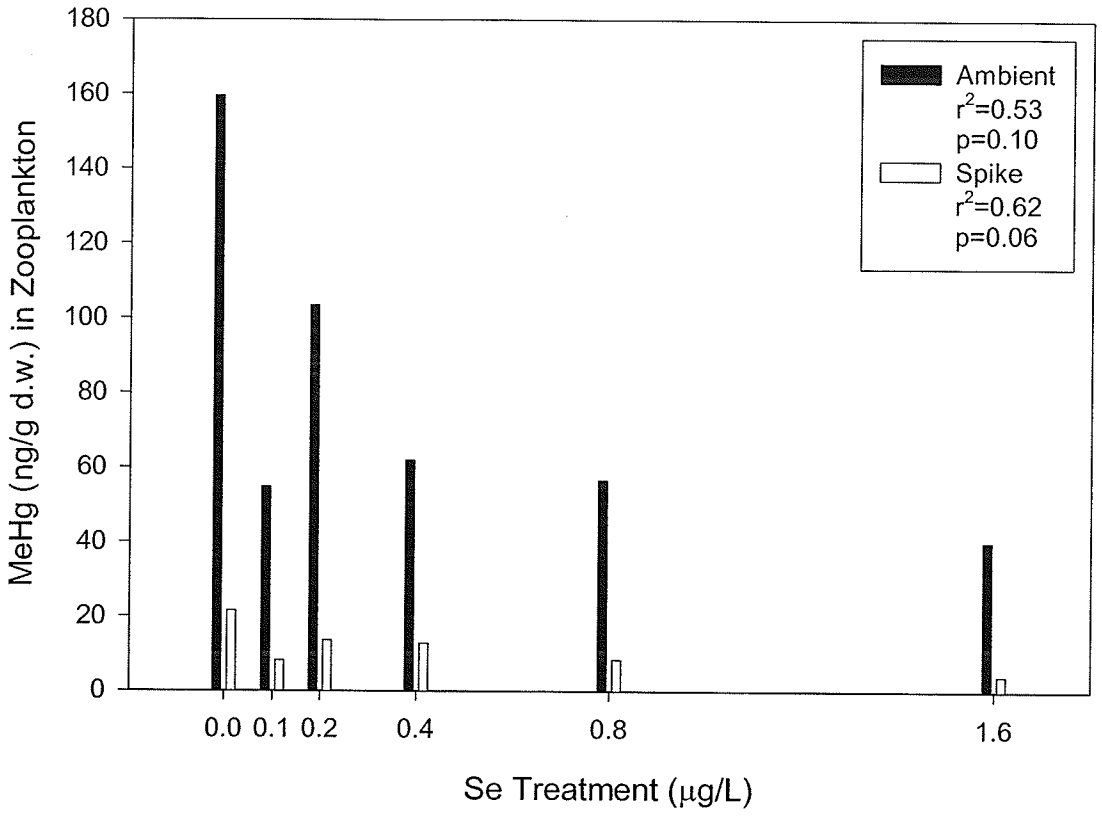


Figure 7. Ambient and spike MeHg concentrations in Chironomid larvae versus Se Treatment in August.

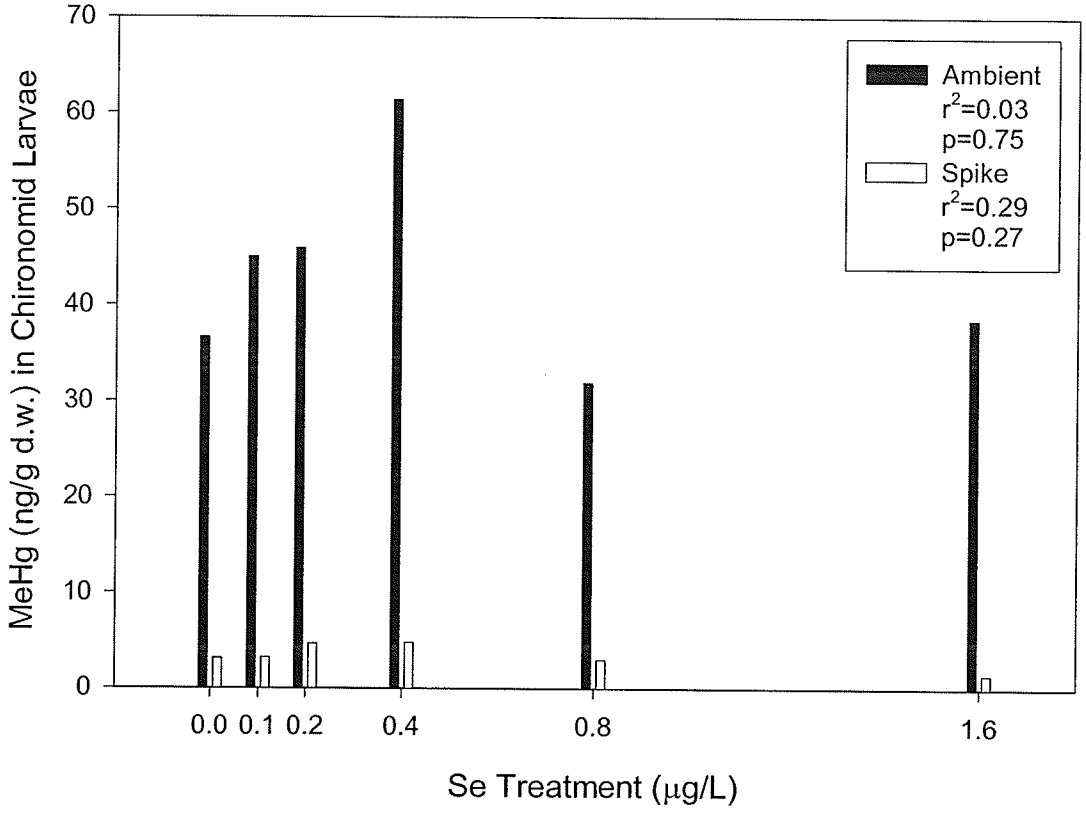


Figure 8. Ambient and spike MeHg concentrations in periphyton versus Se Treatment in August. The error bars are one standard deviation.

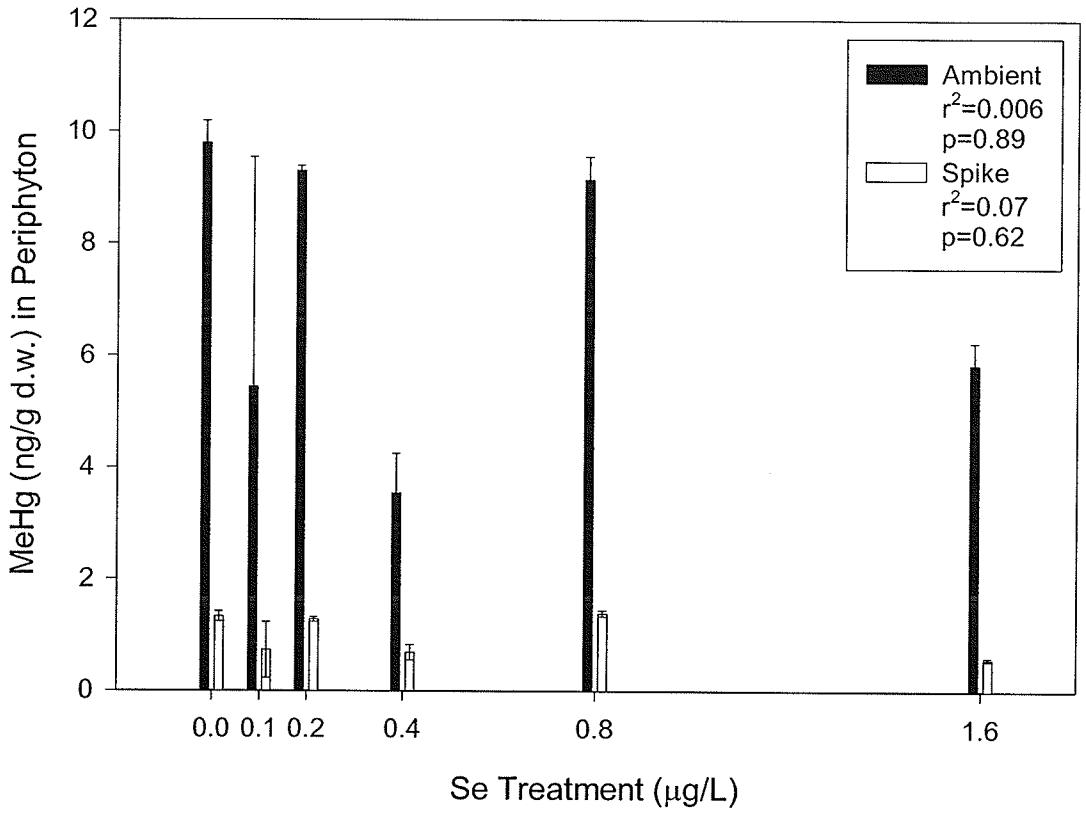


Figure 9. Percentage of the MeHg that occurred as spike MeHg in periphyton, zooplankton, and Chironomid larvae versus TSe concentrations in filtered water in August. Each bar represents one sample for zooplankton and Chironomid larvae, and three samples for periphyton with one standard deviation.

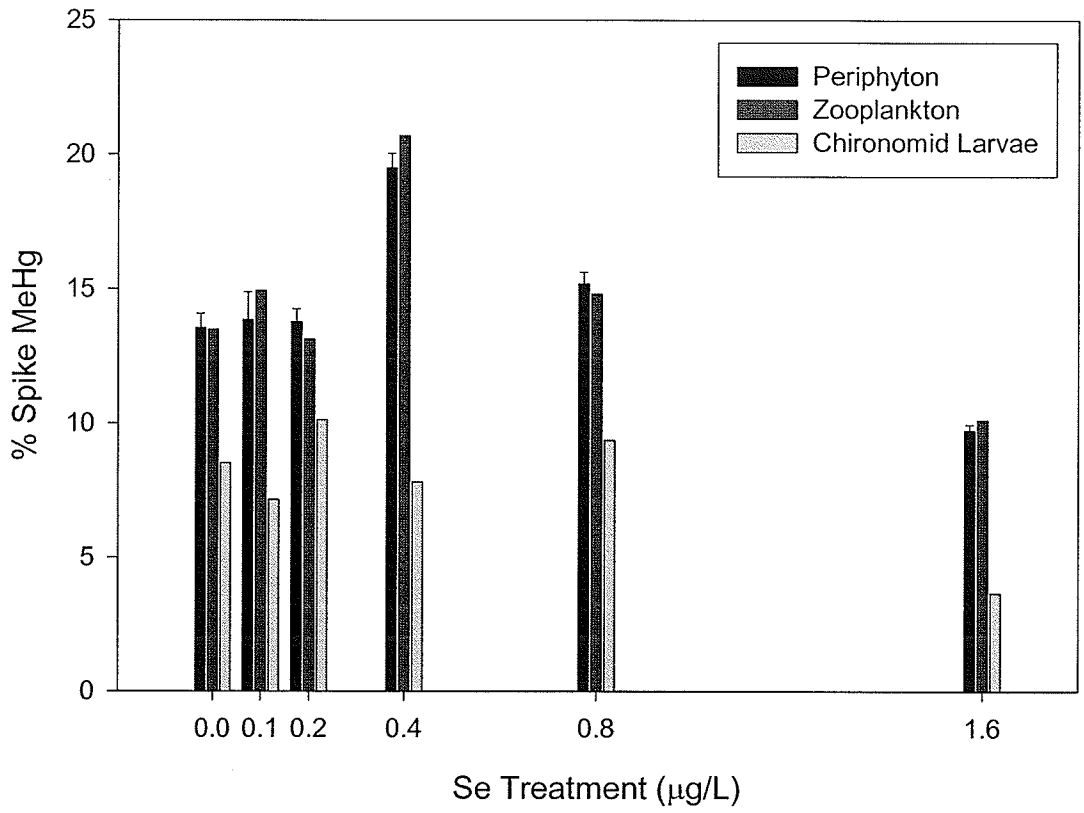


Figure 10. Concentrations of MeHg in muscle of yellow perch versus TSe concentrations in filtered water. Each bar represents the mean with one standard deviation. Sample sizes are noted above groups of bars.

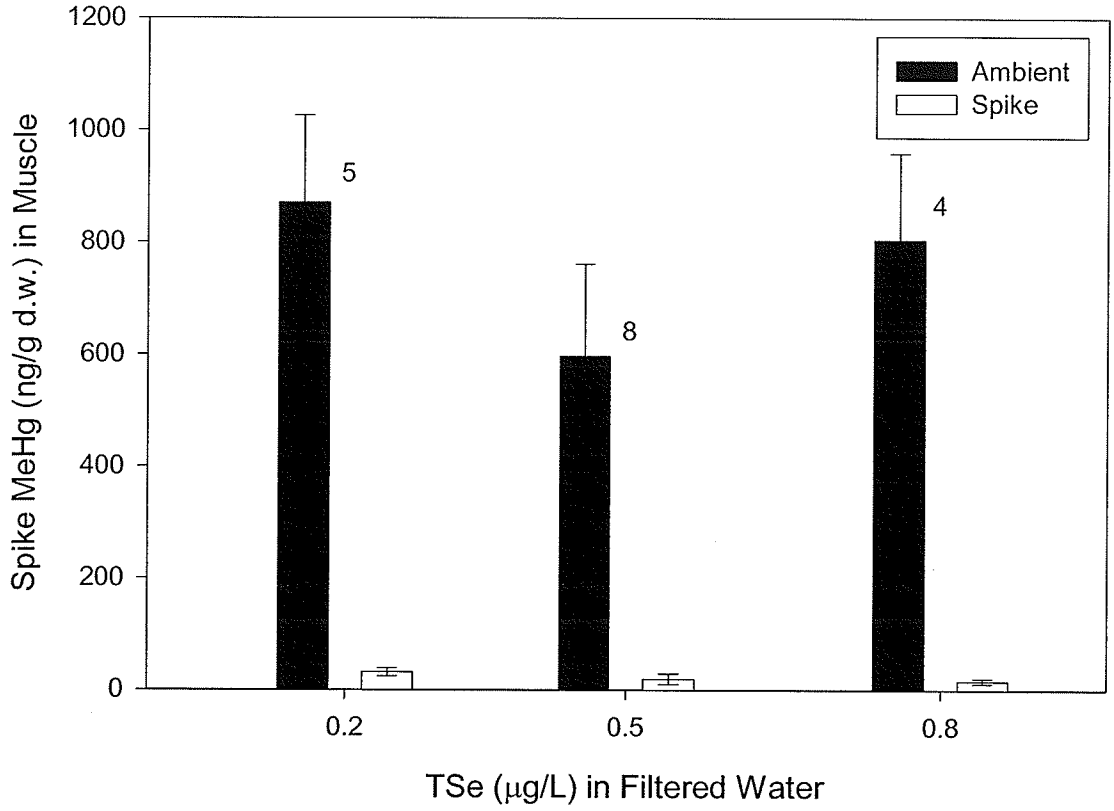


Figure 11. Percentage of THg that occurred as MeHg in muscle of yellow perch versus TSe concentrations in filtered water. Each bar represents the mean with one standard deviation. Sample sizes are noted above groups of bars.

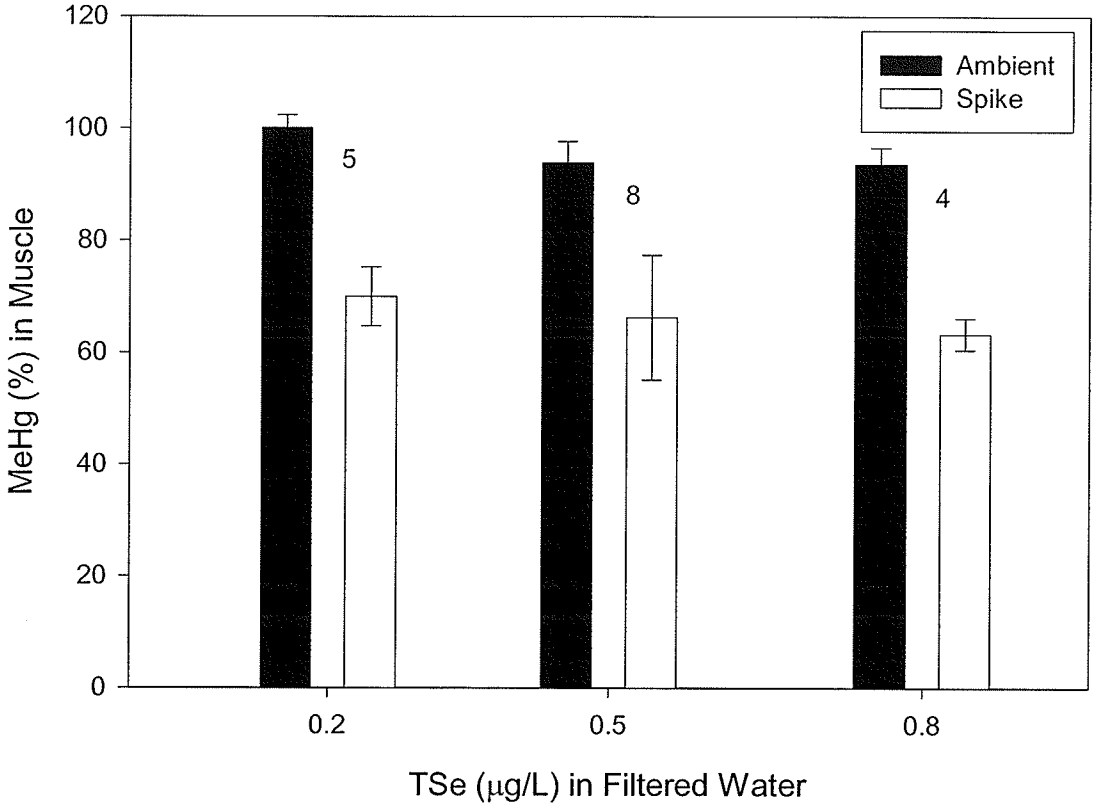


Figure 12. BAFs of spike MeHg in zooplankton, Chironomid larvae, and periphyton as a function of TSe concentrations in filtered water in August. Each data point for zooplankton and Chironomid larvae represents one sample and each data point for periphyton represents the mean of three samples with one standard deviation.

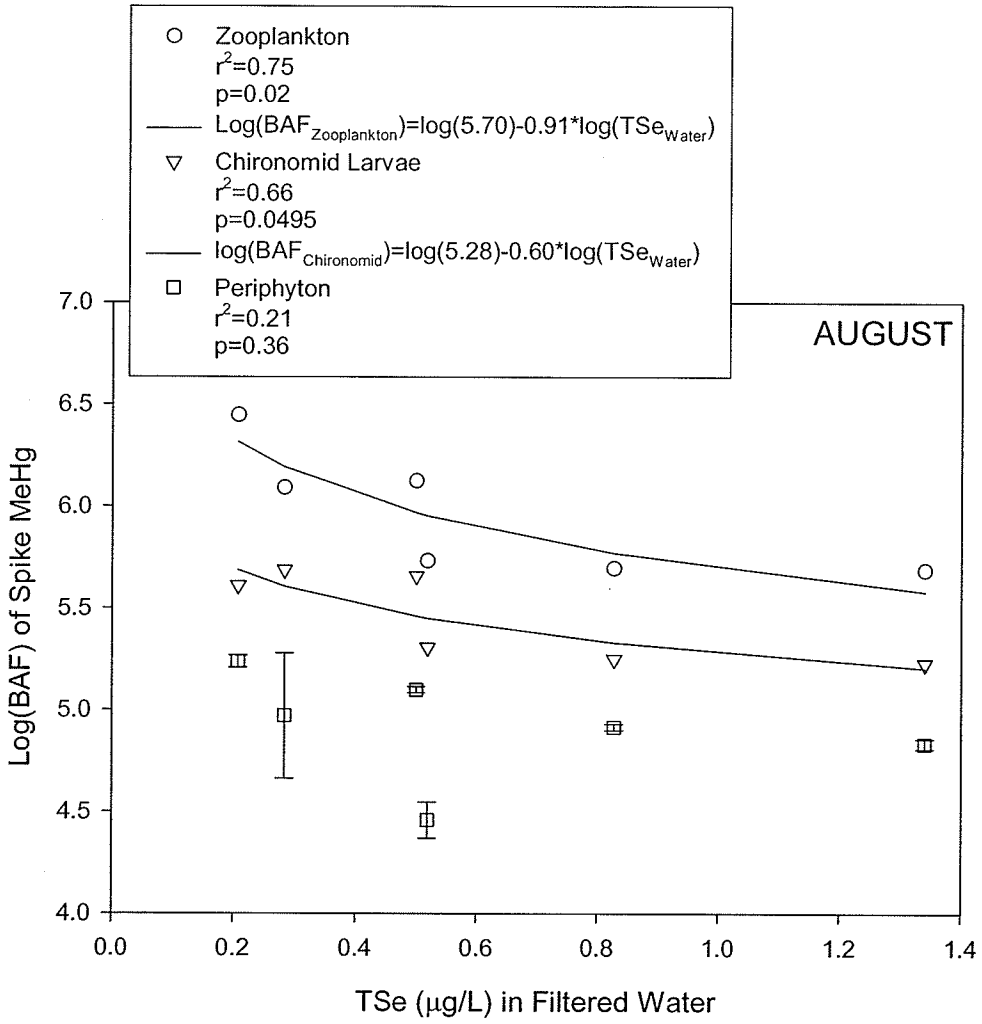
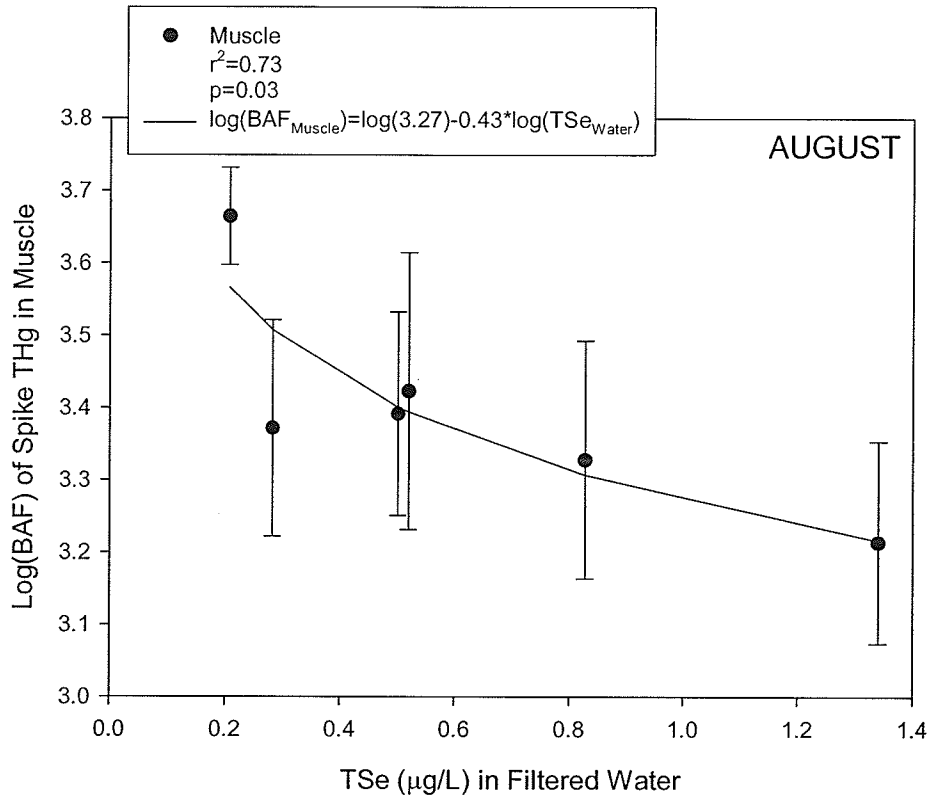
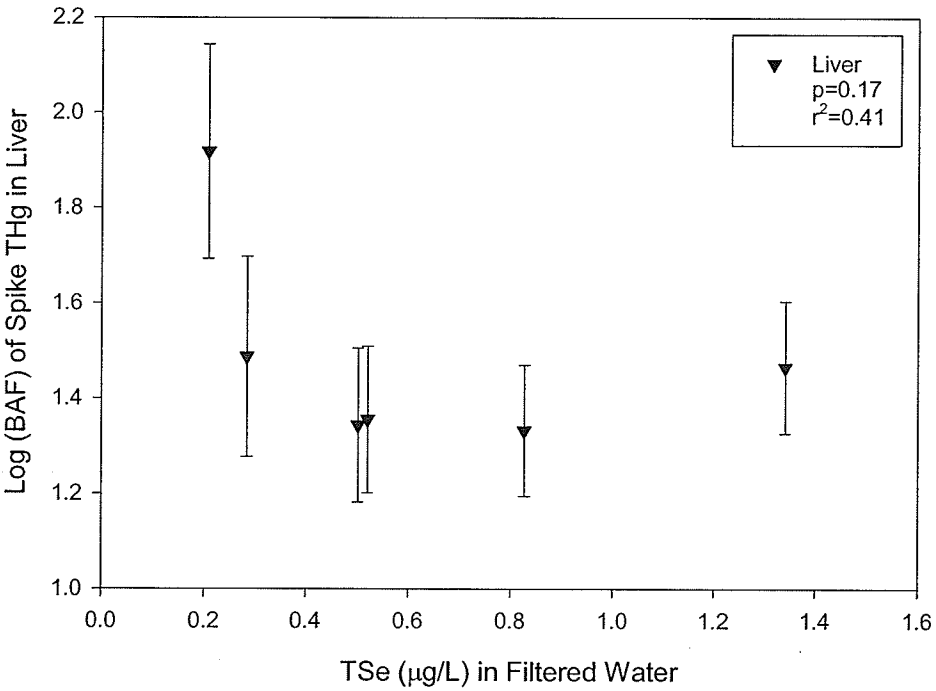
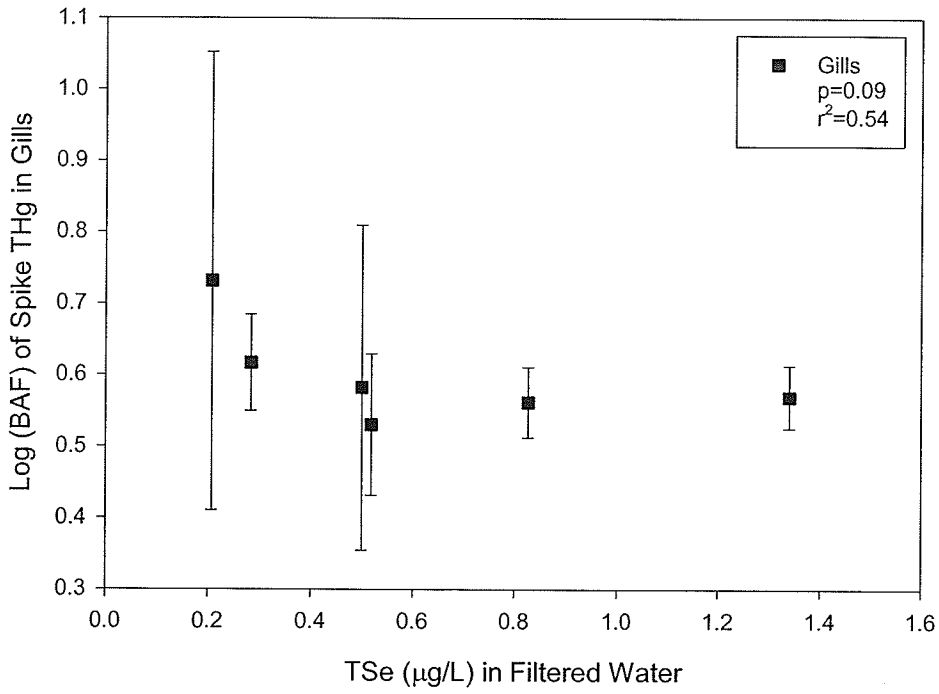


Figure 13. BAFs of spike THg in muscle of yellow perch as a function of TSe concentrations in filtered water. Each data point represents the mean with one standard deviation.






Gills
p=0.09
r2=0.54
Log (BAF) of Spike THg in Gills
TSe (µg/L) in Filtered Water
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6

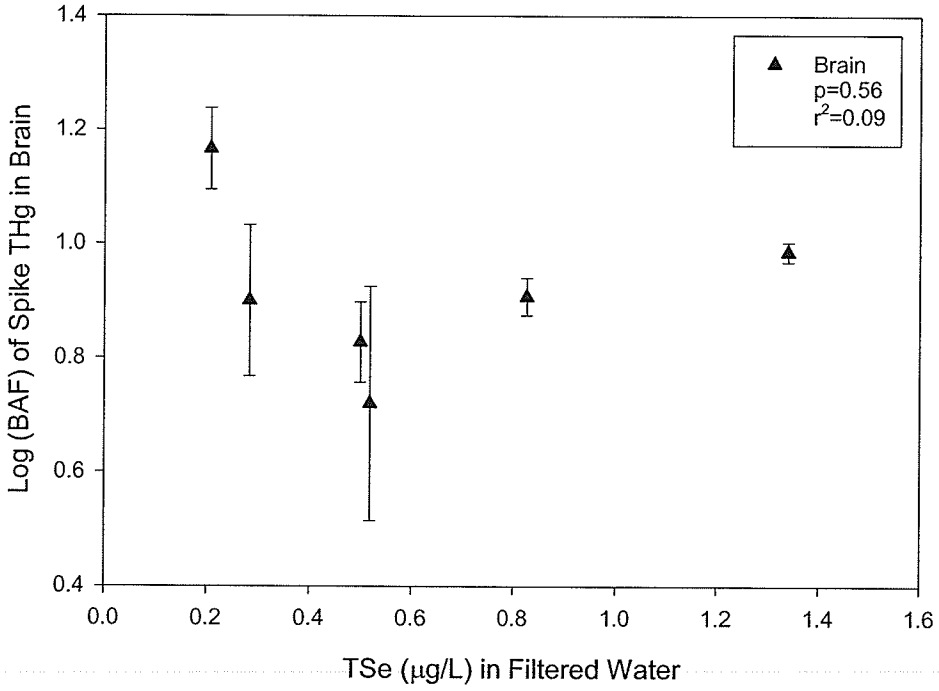

Brain
p=0.56
r2=0.09
Log (BAF) of Spike THg in Brain
TSe (µg/L) in Filtered Water
1.4
1.2
1.0
0.8
0.6
0.4
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6

Figure 14. Pre-treatment (June) data of $\delta^{15}N$ and $\delta^{13}C$ in muscle of yellow perch, Chironomid larvae, zooplankton, periphyton, and surface sediment from each mesocosm. No periphyton samples were collected in June. Yellow perch in June are from the lake that was the source of the fish. Error bars are one standard deviation (periphyton n=3; sediment n=3; yellow perch: n=20). Zooplankton samples for Se Treatment 1.6 µg/L in June were not available.

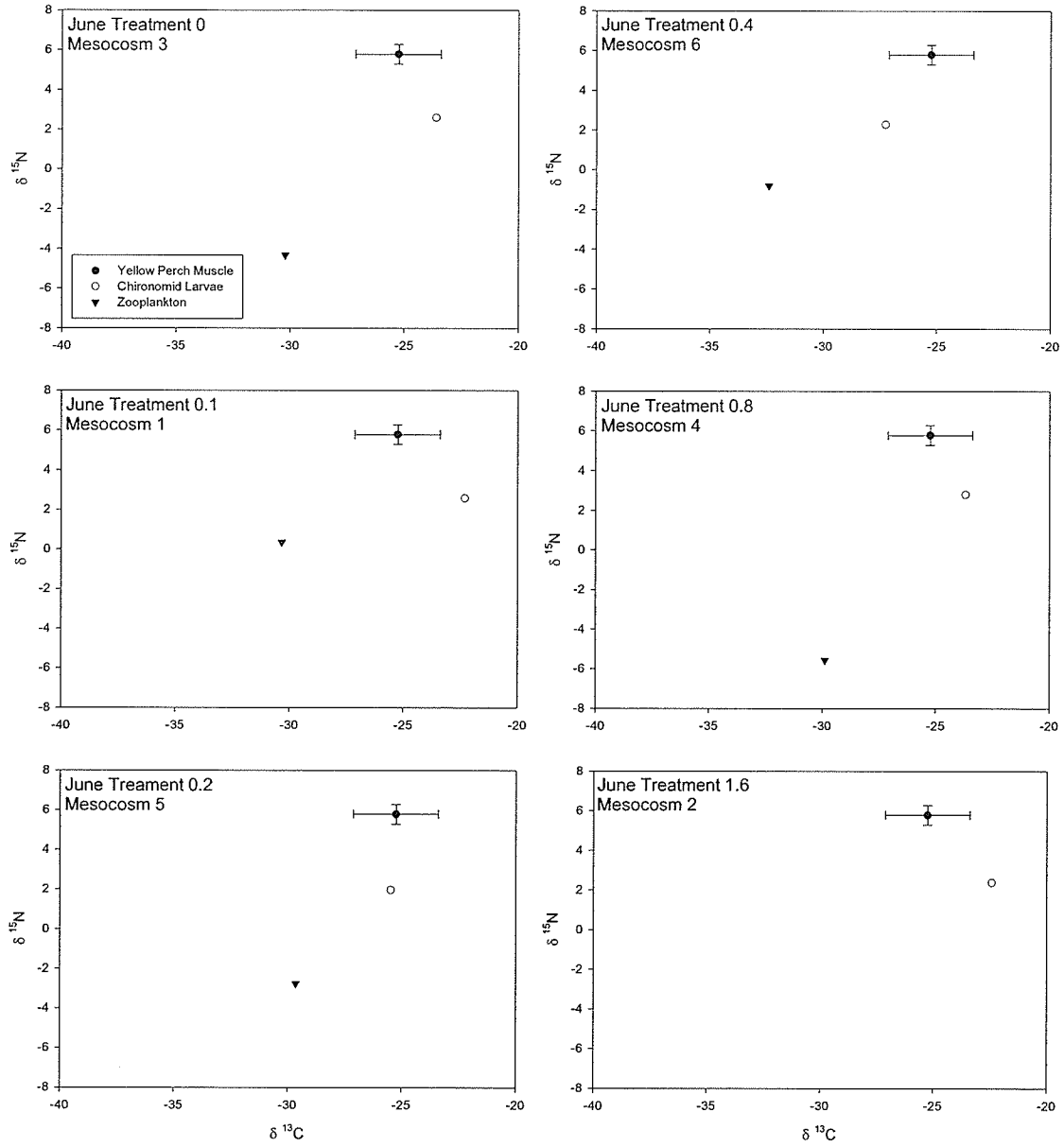


Figure 15. $\delta^{15}N$ and $\delta^{13}C$ values in muscle of yellow perch, Chironomid larvae, zooplankton, periphyton, and surface sediment from each mesocosms in August. Data points represent one composite sample of zooplankton and Chironomid larvae, periphyton (n=3); surface sediment (n=3); muscle of yellow perch (n=8, 2, 5, 4, 9, 16, respectively by mesocosm) with one standard deviation. Solid circles represent muscle of yellow perch; open circles represent Chironomid larvae; solid triangles represent zooplankton; open triangles represent periphyton; solid squares represent surface sediment (0-2 cm).

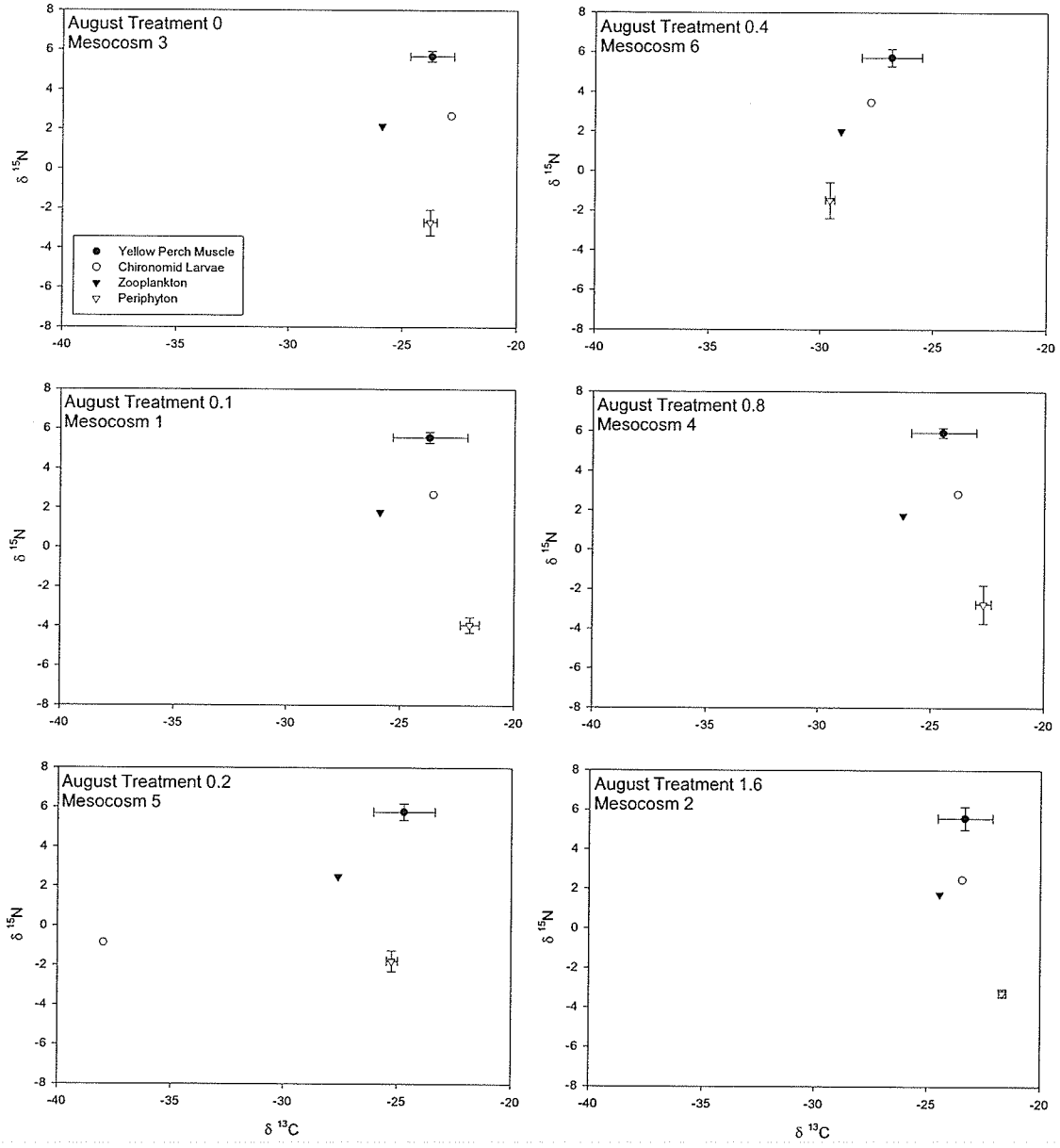


Figure 16. Concentrations of TSe in gonads of yellow perch as a function of TSe concentrations in filtered water. Each data point represents the mean of all measured concentrations with one standard deviation. The suggested Se toxicity threshold for gonads is 10 µg/g (dry weight) while the USEPA threshold for Se in tissue of fish is 7.9 and 5.8 before winter. The sample size is depicted above the error bars.

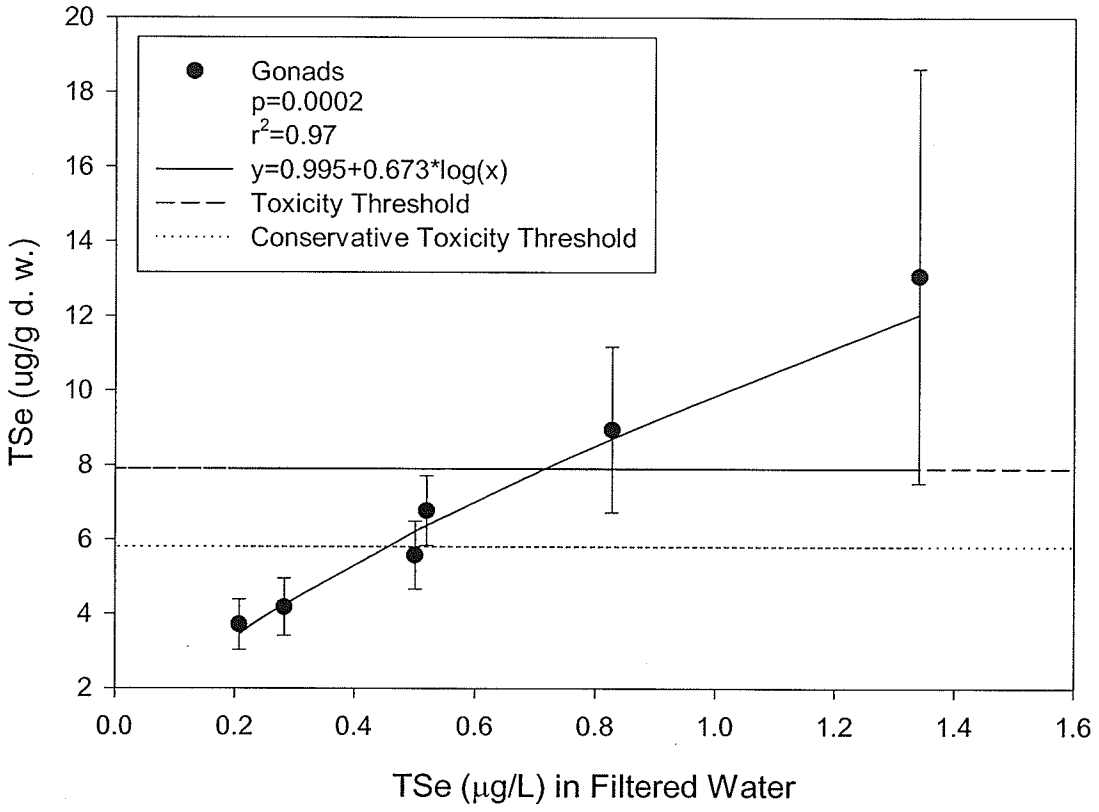
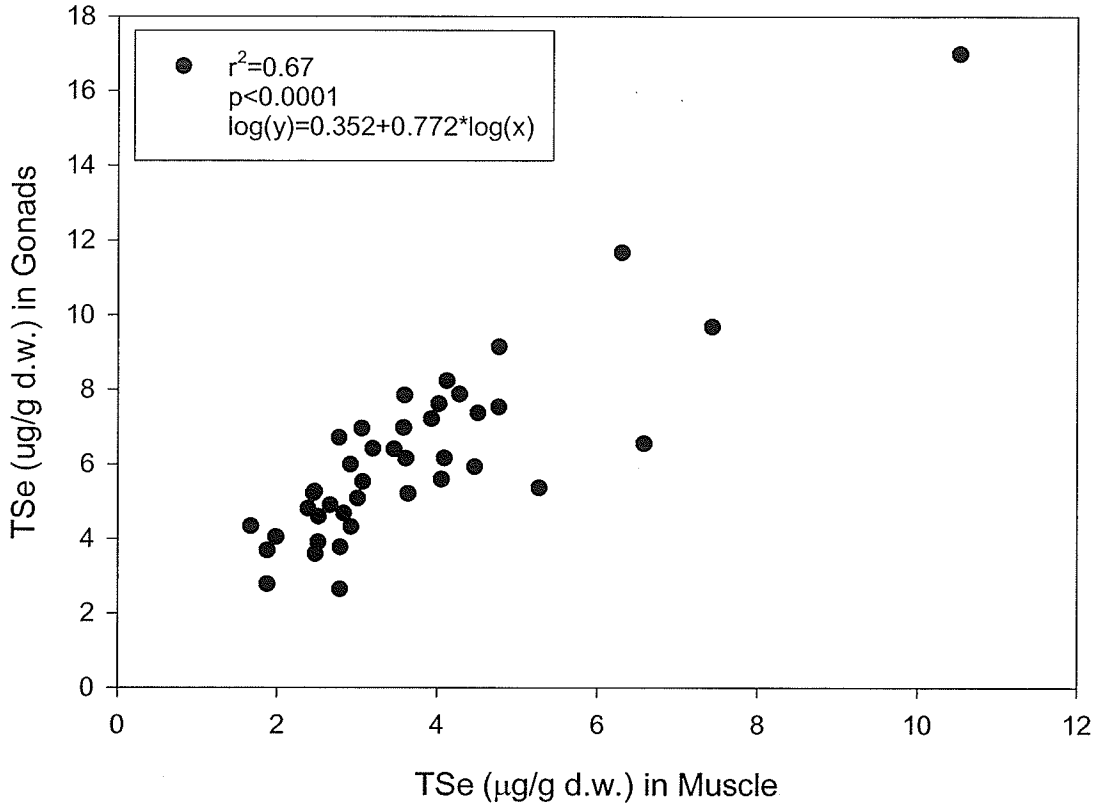


Figure 17. Concentrations of TSe in gonads as a function of concentrations of TSe in muscle of yellow perch. Each data point represents one fish. All fish were collected from mesocosms at the end of our study.



GENERAL CONCLUSION

Although the mechanisms by which Se lowers Hg concentrations in fish and their relative importance are not thoroughly understood, this study provides compelling support for modification of MeHg bioaccumulation; however, it is only one study conducted using relatively low levels of Se. Many studies have documented significant inverse correlations between Hg and Se concentrations in muscle of fish, although the mechanisms by which this occurred are unclear. Regardless of the complexity and specificity of the mechanisms, low-level Se additions lowered Hg concentrations in muscle of yellow perch in this study using mesocosms in a sandy-bottom, circum-neutral, oligiotrophic, freshwater lake.

The effectiveness of the strategy of adding Se to lakes and reservoirs to lower Hg concentrations in fish depends on the extent to which Hg concentrations in muscle of fish are lowered and whether the Hg is redistributed or depurated. Concentrations of Hg should be lowered to below the safe consumption guidelines to enable unrestricted fish consumption and commercial sale of fish. If the Hg is redistributed within fish, humans may have lower Hg exposure, but organisms that eat the whole fish will not. Also, employing this strategy must ensure that Se concentrations in gonads of fish do not become elevated because teratogenesis and possibly recruitment failures in populations of fish could ensue. Results shown here use low Se concentrations and still exhibit a risk of Se toxicity to fish progeny.

In a reconnaissance survey, we examined whether flooding caused concentrations of Se to become depressed in water, zooplankton, benthic invertebrates, and muscle of fish. Flooding causes increased rates of methylation, which may cause Se to be converted to volatile forms that would evade the water to the atmosphere and result in depressed concentrations of Se in reservoirs. Our results did not consistently support depression of Se concentrations in flooded environments. There were lower Se concentrations in reservoirs relative to reference lakes in less than half of the environmental compartments. Factors that affected Se concentrations could be the age of the reservoir, water chemistry, productivity, fish age and growth rate, fish diet and length of the food web, metabolism, climate, spawning, migration, the similarity and proximity of reservoirs and reference lakes that were being compared, and the time of sampling. Reservoirs at the ELA were fed directly from reference lakes whereas those in Québec had reference lakes that were far from the reservoirs. Thus the reservoirs at the ELA are a more direct comparison than those in Québec.

In systems in Québec, water, zooplankton, and invertebrates contained half the Se concentrations as those found in the ELA. Factors contributing to these differences could be the local bedrock, pollution, agriculture, glaciations, water turn-over rates, fish species, fish age and size, productivity, and climate. Also, only total Se was analyzed. Analyzing Se species may have provided valuable insights. However, total Se concentrations were similar to those observed in other studies.

We also evaluated whether changes in Se concentrations were correlated with Hg concentrations in environmental compartments. The results regarding Hg and Se relationships were variable. Concentrations of Hg in fish from the ELA had no relationship with their Se concentrations. There was a positive relationship between Hg and Se concentrations in muscle of fish from Québec. In zooplankton from the ELA, there was a significant negative relationship between MeHg and Se concentrations. The positive relationship found in fish from Québec is contrary to other studies, which usually show a negative relationship. In our study, elevated Se concentrations in muscle of fish were not correlated with Hg concentrations in muscle. In fact, when Se concentrations in muscle were low, Hg concentrations in muscle were also low, but when Se concentrations in muscle were high, Se concentration were either low or high.

The data from this study do not support using Se to lower Hg concentrations in muscle of fish. There was no relationship between Hg and Se in fish from the ELA and there was a positive relationship in those from Québec. Also, all sites except the ELARP reservoir had invertebrates with Se concentrations that met or exceeded the dietary requirements in the diet of fish. The concentrations of Se do not appear to be lower in flooded systems and therefore we cannot recommend adding Se to restore Se that was lost due to flooding.

We tested whether adding Se at different concentrations to mesocosms in a freshwater lake affected Hg or Se partitioning among environmental compartments. The distribution of Hg among compartments was unrelated to Se treatments, except for ambient MeHg concentrations filtered water that were positively related to Se concentrations in filtered water. Thus Se may have enhanced MeHg production. More TSe and Se^{4+} partitioned to particulate matter with increasing Se concentration in water. However, less Se^{6+} partitioned to particulate matter with increasing Se concentrations dissolved in water. The greatest sink for spike THg was the unmeasured portion, which we assume to be the atmosphere. The next most important pools were surface sediment, filtered water, and then particulate matter. The half-life of spike THg

dissolved in water was 26 to 36 days. For Se, the greatest mass was found in surface sediment and then filtered water, but we could not distinguish between added and ambient Se and thus this does not indicate its fate. Our results agree with other studies that added Hg and Se to lakes or mesocosms. The stability of Se in water may indicate repeated additions would not be necessary, but in whole-lake systems this would depend on flushing rates, circulation patterns, and stratification.

We used regression analyses to examine whether Hg or MeHg concentrations in aquatic organisms were related to Se concentrations in filtered water. Concentrations of Hg in the muscle and liver of yellow perch and the BAFs of Hg in muscle of fish were inversely related to the Se concentrations in filtered water. About 65% of spike Hg in muscle was present as spike MeHg. Also, the BAFs of MeHg in zooplankton and Chironomid larvae were inversely related to Se concentrations in filtered water. It seems likely that Se lowered Hg in fish and the lower food web by modifying either uptake or retention of Hg. There was more spike Hg in liver than gills of fish, which may support greater uptake via diet than from water. Also, there was much more spike Hg in liver than muscle, which may indicate Hg first entered the liver and was then transferred to the muscle. Further, it appears Se decreased the amount of Hg in the liver and thus decreased the amount of Hg that could be transferred to the muscle. Because these systems were not at steady state, they could have become even lower with time.

We asked whether Se could effectively lower Hg concentrations in muscle of fish without imposing toxicity to those fish. We assessed whether concentrations of Se in the gonads of yellow perch were below the most widely accepted toxicity threshold of 7.9 μg/g (5.8 before winter). Again, using the regression equation, concentrations of 0.7 μg/L of Se in filtered water resulted in about 8 μg/g of Se in gonads and 0.5 μg/L resulted in about 6 μg/g of Se in gonads. Gonads are the most appropriate tissue to analyze for Se concentrations because the endpoint of chronic Se toxicity is in the offspring. Because these systems were not at steady state after eight weeks, Se concentrations in gonads could have become more elevated with longer exposure. It seems unlikely that less than 0.5 to 0.7 μg/L of Se added to freshwater would be able to effectively lower Hg concentrations in muscle of fish without a risk of chronic Se toxicity to fish. For longer term studies on larger fish, Se would need to be monitored in gonads of fish.

One further finding was a positive relationship between concentrations of TSe in muscle and gonads of yellow perch. Therefore Se concentrations in gonads can be estimated by measuring Se concentrations in muscle within the range of concentrations and lengthe of fish that we reported. Thus non-lethal sampling methods could be employed to monitor Se

concentrations in gonads of fish to assess their risk of Se toxicity assuming this regression holds for longer periods of time.

There are benefits and risks associated with adding Se to lower Hg concentrations in muscle of fish. Additions of Se are effective, inexpensive, and fairly easy to apply, would be informative, and could be applied to select water systems. The risks are the need for repeated additions, accumulation in sensitive habitats that fish and birds use as nurseries, Se toxicity and failure to recruit, meeting target concentrations of Se with seasonal fluctuations and winter stress to fish, the cost of monitoring, and setting the precedence for other chemicals to be added. We found prey items of fish already met and exceeded Se concentrations in the diet of fish, even in regions and in hydroelectric reservoirs with very lower Se concentrations in water. We also found concentrations of Se were not depressed in reservoirs and adding Se to supplement to natural background concentrations is not advised. Low-level additions of Se exceeded the threshold for Se toxicity in gonads of fish in our study. Our results indicate Se should not be added to lakes or reservoirs at any concentration.

To comprehensively assess the viability of adding Se to lower Hg concentrations in fish, we suggest some guidelines for future studies. Environmentally relevant concentrations and chemical forms of Hg and Se should be used. The route of exposure in nature is mostly via diet and minimally through water, and thus water and dietary exposure should be used in laboratory experiments. Currently, monitoring the appropriate endpoint of Se toxicity is to monitor Se concentrations in gonads of fish. In addition, liver and muscle should be monitored and tissues of fish should be dried before analysis to prevent variation due to water content. Analysis of MeHg in tissues of fish is important because Se has been shown to affect MeHg and THg differently. The possibility that Se increases Hg loss from fish could be investigated in the field using fish that were previously exposed to an isotope of Hg and then exposed to different amounts of Se through their diet.