Comparing Mercury, Arsenic, and Selenium in Several Fish Species from Two Nova Scotia Lakes

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Abstract

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The environmental increase of mercury and arsenic - two contaminants with no biological functions - due to anthropogenic activities is a complex and growing issue. Both mercury and arsenic, in particular methylmercury and inorganic arsenic, can bioaccumulate in the food web. Exposure to elevated concentrations of either can result in serious potential human and wildlife health impacts. This can be further complicated by either the presence or absence of selenium, which is an essential micronutrient. Se can result in health impacts for both humans and wildlife in excess or in deficiency. Selenium is of importance due to its antagonistic relationship with mercury, but in Nova Scotia, water, sediment and soil Se concentrations are typically quite low. For this study, mercury, arsenic and selenium concentrations were analyzed in fish tissue between Shortts Lake (Colchester County), which had low groundwater and sediment arsenic levels, and Morris Lake (Halifax County), which had high groundwater and sediment arsenic levels. The four fish species of interest were: *Alosa pseudoharengus* (gaspereau or alewife), *Micropterus dolomieu* (smallmouth bass), *Esox niger* (chain pickerel), and *Morone americana* (white perch).

Mercury and arsenic concentrations in many individuals of all four species from both lakes exceeded the World Health Organization's (WHO) daily tolerable intake limit of 0.0177 mg/kg, and 0.233 mg/kg respectively. The trend between total mercury concentration of fish tissue and the length of fish resulted in a positive correlation in both lakes. Shortts Lake fish tended also had higher total mercury concentrations than those from Morris Lake. Total arsenic concentration did not significantly vary amid fish of the same species between lakes, but gaspereau (alewife) was found to have significantly higher total arsenic concentrations in both lakes compared to the other three fish species. Selenium concentrations did not significantly differ between same species of different lakes, nor between lakes.

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Background

Introduction to Mercury and Its Sources

Mercury (Hg) is a naturally occurring element that, when not disturbed, is found mainly sequestered in rock formations and soil. These sources can be emitted into the atmosphere by volcanism, as well as by natural disruptions of the rock source via erosion and weathering (Gustin & Lindberg & Wesberg, 2008). While mercury maintains a complex relationship with other environmental compounds, there are three major mercury species: elemental (Hg^0), inorganic (Hg^{2+}), and methyl mercury (MeHg) (O'Driscoll & Rencs & Lean, 2005). Due to its volatility, Hg^0 is the main species found in the atmosphere. Once in the atmosphere, Hg is transformed via oxidation into its soluble species, Hg^{2+}, which then gets deposited into surface waters where Hg^{2+} binds with organic and inorganic molecules (O'Driscoll et al., 2005). The methylation process of mercury from Hg^{2+} to MeHg can occur in anoxic (e.g., lack of oxygen) and more acidic conditions that encourage the presence of methylating bacteria and other microorganisms (Gochfeld, 2003; Wiener, 2013; Vaidya & Howell & Leger, 2000).

Over the past 100 years, anthropogenic activities have increased the global atmospheric mercury pool through mining, coal burning and industrial processes (O'Driscoll et al., 2005). And since the beginning of the industrial revolution, it has been estimated that atmospheric mercury pool has increased ten-fold (Zhang & Wong, 2007). One major contributor to atmospheric Hg is emissions from the burning of fossil fuels and biomass fuels to produce energy (Sunderland & Chmura, 2000). Other anthropogenic sources include medical waste incineration, dental amalgams, pharmaceuticals, fluorescent bulbs, base-metal smelting, pulp and paper plants, and gold mining operations (Sunderland & Chmura, 2000; Driscoll et al., 2007).

Due to mercury's long half-life in the atmosphere (one to two years), mercury can be deposited far from the original source. Therefore mercury contamination is a global issue, and has resulted in atmospheric deposition in regions that historically are not prone to elevated mercury concentrations (Sunderland & Chmura, 2000). In Canada, mercury levels taken from lake sediments have revealed to be on average two to three times the background level (Vaidya et al., 2000). Atlantic Canada in particular, has elevated mercury levels partly due to its location downwind from major air pollutant emission areas throughout Eastern North America. This has resulted in a concentration of pollutants, acid rain, acidification and methylation of deposited mercury in the freshwater ecosystems of the Maritimes (Vaidya et al., 2000).

In Nova Scotia, an important historical source of mercury contamination is gold mines, which were most active between 1860 and 1945 (Wong & Gauthier & Nriagu, 1999). Mercury was commonly added to crushed ore, which would then create an amalgam that dissolves the gold and hence provided a means to separate the gold from the ore and silt (Zolnikov, 2012; Veiga & Maxson & Hylander, 2006). Once the gold and mercury rich amalgam was removed from the ore, it was then heated to burn off the mercury (which added to atmospheric Hg levels), leaving the gold behind (Veiga et. al., 2006). Due to the widespread use of liquid mercury to extract gold ore, it is estimated that 6800kg of Hg has been left in tailing piles. Any excess mercury was left in tailing piles; historically in Nova Scotia, these tailing piles were not properly secured, covered or contained, which allowed the mercury to leach into the surrounding environment, soil, aquifers, and water bodies via a combination of seepage, run-off, weathering and erosion, and wind mobilization of contaminated sediment (Wong et al., 1999). Furthermore, these previous anthropogenic Hg releases may be a significant source of ongoing contamination; Hg is recycled back into the environment as it is unable to biodegrade, and therefore mine

tailings over 100 years old still pose a significant concern to this day (Sunderland & Chmura, 2000). Gold is widely distributed within the Meguma Terrane, which stretches from the southern tip of Nova Scotia, to the Cobequid-Chebucto Fault, and as a result, hazardous mining wastes are found throughout this region (Ryan & Smith, 1998). In this particular study, the lakes were selected to be distant from direct impacts from historical gold mines although it is feasible that regional gold mine waste sites could still be an indirect source (Government of Nova Scotia, 2014).

Bioavailability, Bioaccumulation and Biomagnification of Mercury

Bioavailability indicates a substance's availability to be absorbed into the body and have an active effect (Environment Canada, 2013). *Bioaccumulation* is the build-up of a substance, such as Hg, within the tissues of a biological organism, and *biomagnification* is the increase of the concentration of a substance within organisms (from food sources) with the increase of trophic level in food chains (Environmental Protection Agency, 2010; Environment Canada, 2013).

Each mercury species has different properties, routes of exposure and bioavailability. Hg^0 vapour can be readily bioavailable via inhalation, but is poorly absorbed via the gastrointestinal (GI) tract and skin contact, while conversely MeHg is highly absorbed via the GI tract (Gochfeld, 2003). Hg^{2+} absorption rates varies greatly depending on its environment and the compound with which it binds. But it is MeHg that has the highest toxicity, the greatest bioavailability to humans and animals, and the uppermost ability to bioaccumulate and biomagnify, and therefore is of greatest concern (Gochfeld, 2003).

Bioaccumulation and biomagnification can result in fish having MeHg concentrations a million-fold greater than the water in which they live (Gochfeld, 2003). Due to MeHg high bioavailability, tissues of higher trophic animals typically have proportions of 90-95% MeHg and 5-10% other Hg speciations (Gochfeld, 2003; Wiener & Krabbenhoft & Heinz & Scheuhammer, 2003). In lower trophic levels, MeHg's proportion is much lower, but the comparison between levels demonstrates MeHg's ability to biomagnify; the average proportion for MeHg in aquatic environments is 5%, which then magnifies to 15% in phytoplankton, 20% in zooplankton, and greater than 90% in fish and other higher order trophic species, such as humans (Crump & Trudeau 2009). Therefore, total mercury (THg) in fish tissues – indicating the total amount of all mercury compounds analyzed - mostly consists of MeHg. Due to the low cost of THg analyses relative to MeHg analyses and the ease of rapid high-flow-through analyses in the laboratory, many scientific studies, including this one, use THg in place of MeHg in tissue (Wiener et al., 2003).

Health Impacts of Mercury

Mercury is one of the most toxic metals found in the environment, and lacks any biological function. For humans, the most common routes of exposure include inhalation, consumption of water, fish and food items, and ingestion of soil. Exposure to mercury has been found to have human health impacts, even at low doses (Zhang & Wong, 2007; Singh et al., 2011; Karagas et al., 2012).

Once exposed to Hg, the behaviour of Hg within the body is dependent on its speciation and route of exposure. Eighty percent of inhaled Hg^0 is absorbed, which then accumulates in the

kidneys and is distributed to all parts of the central nervous system as a lipid-soluble gas, or it is converted into inorganic Hg which attaches to certain proteins, such as enzymes, glutathione, and most structural proteins (Ozuah, 2000; Clarkson, 2002). This results in denaturing these proteins, and inhibiting the functions of the respective enzymes (Clarkson, 2002). At this state it also has the potential to cross the blood brain barrier (BBB), or to reach the placenta. However, if Hg^0 is ingested, the majority of it is excreted in the feces (Ozuah, 2000; National Research Council, 2000).

If Hg is ingested as MeHg, such as when contaminated fish tissue is consumed, 95% of it is absorbed, which is then distributed to all tissues throughout the body within 36 hours (Ozuah, 2000; Clarkson, 2002). MeHg is particularly adept at crossing the BBB and the placental barrier, and hence can be found in concentrations five to seven fold higher in the fetal brain than in the maternal blood (Charleston et al., 1996). This tendency is of concern regardless of life stage, but is particularly concerning during development, as MeHg preferentially collects on astrocytes - cells that form myelin, and provide support and protection for neurons – and hence disrupts or degrades neurological functions, interrupts membrane transport, can cause cerebral palsy from birth, and extreme fetal abnormalities (Aschner, 1996; Eto, 2000; Clarkson, 2002; Karagas et al., 2012).

Exposure can result in acute or chronic symptoms. Acute symptoms develop three to five hours after high exposure and can manifest in coughing, lethargy, and fever (Landford & Ferner, 1999). Chronic symptoms often manifest as a triad of increased excitability and irritability, tremors, and gingivitis. But it also can cause central and peripheral nervous system damage, fine tremors of the extremities and facial muscles, constant emotional change, irritability, pink

disease (a pink rash on the extremities), severe itchiness, and chronic pain (Tunnessen et al., 1987; Ozuah, 2000; Singh et al., 2011).

In aquatic ecosystems, elevated exposure to Hg can be toxic to benthic communities, and cause cellular damage to place plants, therefore resulting in a loss of habitat and negatively affecting fish populations (Wong et al., 1999; Singh et al., 2011). Mammals, fish, and birds undergo decreased reproductive success, impaired hormone production, behavioural changes, and potentially even death (Mulligan et al., 2001; Scheuhammer et al., 2007; Akearok et al., 2010; Singh et al., 2011). In fish, Hg acts as an inhibitory at multiple reproduction sites including the hypothalamus, pituitary, and gonads, while also acting as a neurotoxin causing neuron degeneration, and alterations in neurotransmissions (Crump & Trudeau, 2009). Regardless of the organism or Hg speciation, heighten exposure can cause severe health impacts to humans, plants, and wildlife alike.

Introduction to Arsenic and Its Sources

Arsenic (As) is a naturally occurring element that is ubiquitous in the environment. The most common natural sources are volcanism, rock formations, hydrothermal ore deposits, and the water runoff from weathering of the As-containing rocks (Rodie et al., 1995; Wang & Mulligan, 2006). Arsenic can be found in four oxidation states, with nearly twenty respective As species, which range in toxicity and mobility (Rodie et al., 1995; Wang & Mulligan, 2006; Wang et al., 2014). However, in the environment, As is primarily found as inorganic arsenate (As(V)), and arsenite (As(III)), which are among the most toxic forms (Wang et al., 2014). The majority of arsenic is sequestered in rocks, and it is via weathering and erosion processes that mobilize and transport inorganic arsenic into sediment, soils, and water providing exposure sources for

biota (Rodie et al., 1995; Wang & Mulligan, 2006). These inorganic forms can then be chemically or biologically methylated under anoxic conditions into various organic As species. This includes into volatile forms that are found in the atmosphere, which can be re-deposited into surface waters and soils and once again absorbed by biota (Wang & Mulligan, 2006; Wang et al., 2014).

Anthropogenic sources of arsenic include As-containing herbicides, fertilizers and pesticides, fossil fuel burning (in particular coal burning), metal smelting, gold mining, glass-production, wood preservation, waste incineration and steel-production (Rodie et al., 1995; Matschullat, 2000; Reash, 2012). Due to these anthropogenic activities, the global atmospheric load for As has increased by an estimated factor of 1.6, with 60% attributed to coal combustion and copper smelting (Mason et al., 2000; Matschullat, 2000). These emissions also allow for moderate ranged transportation given that atmospheric As retention is seven to ten days (Matschullat, 2000).

In Nova Scotia, there are four coal-burning power plants located in Lingan, Point Aconi, Point Tupper and Trenton, which generates 60% of Nova Scotia's electrical power, and as of 2014 has contributed a total of approximately 83 kg provincially of As into the air and water via emissions (Nova Scotia Power, 2015; Environment Canada, 2014). These coal-burning power plants have also created, as of 2006, a total of approximately 21000 kg provincially of As in on-site tailing and waste rock disposals (Environment Canada, 2014).

Arsenic is also commonly found in Nova Scotia groundwater and sediments associated with the Meguma geological Terrane, which contains significant amounts of arsenopyrite (Figure 1); this has resulted in elevated concentrations of As in well water and sediments in areas that correspond with the Meguma Terrane (Chappells et al., 2015). This has been further exacerbated

at historical mining sites, which have evacuated and processed significant amount of ore and often are associated with significant elevated As contamination, along with the Hg contamination, from the ore in mining tailings. As a result of the As and Hg, many historical Nova Scotia mining sites are considered hazardous waste sites (Saunders et al., 2010).

Health Impacts of Arsenic

The toxicity of As is dependent on its speciation, with arsenite (As(III)) being approximately ten times more toxic and mobile than arsenate (As(V)) (Wang & Mulligan, 2006). Furthermore, in general, the inorganic forms of As are more toxic than the organic forms. The bioavailability of As compounds are influenced by numerous factors such as the concentration and speciation of As, the type of organism being exposed, environmental conditions affecting the chemistry of arsenic (such as pH), and the presence and concentration of competing ions, such as phosphorus (Wang & Mulligan, 2006).

As(III) is found in anoxic conditions, As(V) is found in oxic conditions, while methylated As species require microorganisms for biomethylation. Once methylated, As becomes particularly toxic as it is transported in the phosphate chain and then efficiently breaks down or damages DNA (Wang & Mulligan, 2006).

In humans, As has been categorized as a group-I carcinogen, and with primary exposure through ingestion of food and drinking water (World Health Organization [WHO], 2010; Li et al., 2014). Chronic exposure can lead to hyperpigmentation and hyperkeratoses on the hands and feet, cancers of the skin, bladder, liver, kidney, and lung, an increased risk in diabetes, and peripheral vascular disease, also known as “blackfoot” disease (WHO, 2010; Gabos et al., 2010; Wang et al., 2014; Li et al., 2014). Acute symptoms of As poisoning include vomiting,

abdominal pain and diarrhea, numbness of extremities, muscle camping, and in extreme cases death (WHO, 2010).

Arsenic is also a known toxin for plants and animals, and exposure to elevated concentrations in soil can result in root depression in plants, and growth defects in plants and animals (Matschullat, 2000; Wang & Mulligan, 2006). Primary As exposure routes in fish are via bioaccumulation through food chains, As particulates suspended in water, absorption into the blood stream by membranes (such as gills), or via adsorption on tissue and membrane surfaces (Shah et al., 2009). The bioaccumulation of As in fish could potentially lead to behavioural changes, and disrupt hormonal, and metabolic processes, as well as death (Sopinka et al., 2010; Shah et al., 2009).

Introduction to Selenium and Its Sources

Selenium (Se), similar to Hg and As, is a naturally occurring element. Unlike Hg and As though, Se is an micronutrient used in numerous essential biological functions (Chapman et al., 2010; Sun et al., 2014). Se is essential to all aerobically respiring organisms, and plays fundamental roles in antioxidant defence, and hence reduces the degradation of cell membranes (Flueck et al., 2012). Common natural sources of Se are rock formations (such as shale, phosphate rocks and coal), volcanism, weathering and erosion of source rocks, wildfires, and volatilization from plants (Chapman et al., 2010). These sources redistribute Se into water, soil, and air, while transforming Se into difference species and phases. In aquatic ecosystems Se is generally in the inorganic oxidation states selenate (Se^{+4}), and selenite (Se^{+6}). Se^{+4} and Se^{+6} are taken up by aquatic microbes, algae, and plants, and internally converted to organic Se compounds, which bioaccumulates and transfer through the food chain via predation on lower

trophic levels (Hamilton, 2004; Chapman et al., 2010; Sun et al., 2014). To a lesser extent, fish and other aquatic wildlife are also known to uptake Se via gills or epidermis from the surrounding water (Hamilton, 2004). Furthermore, the bioavailability is heavily dependent upon the chemical form of Se, of which there are many (although organic forms generally are more bioavailable than inorganic), and the presence of other compounds such as metals, or certain trace elements (Navarro-Alarcon & Cabrera-Vique, 2008; Chapman et al., 2010).

Anthropogenic sources of Se include fossil fuel burning, oil refining, and fertilizers; but other anthropogenic activities such as agricultural irrigation, and mining can mobilize Se from natural sources (Hoffman, 2002; Chapman et al., 2010). In Nova Scotia, Se concentrations in soil are within the global norm (0.4 – 2.0 μg/g), including in areas that are nearby the historical coke oven and smelting operations (CCME, 2009). Nova Scotia water Se concentrations are generally extremely low. In a report by CCME (2009) it was stated that out of the 60 rivers tested in Nova Scotia and New Brunswick all samples were below the detection limit of 1.0 μg/L, with the exception of two samples that were found to be less than 1.4 μg/L. And this is still well below the provincial maximum acceptable concentration in drinking water of 0.01 mg/L (Nova Scotia Department of Environment and Labour, 2008).

Health Impacts of Selenium

Because Se is a micronutrient, the health impacts of Se can either be caused due to deficiency or excess, although the margin between either circumstance is narrow (Navarro-Alarcon & Cabrera-Vique, 2008). In humans, chronic exposure to excess Se is associated with nail and hair loss, gastroenteritis, and dermatitis; while acute exposure acts as a neurotoxin and

can lead to dizziness, fatigue, irritability, paresthesias, dermatological lesions, and, in extreme cases of exposure, death (Bella et al., 2010; Vinceti et al., 2014). Due to the complexity of Se, different speciations vary greatly in their effects as a neurotoxin (Vinceti et al., 2014).

Excess of Se in vertebrates, such as birds and fish, can cause reproductive impairment, mortality, teratogenesis, mass wasting in adults, reduced juvenile growth, and immune suppression (Hoffman, 2002; Chapman et al., 2010). However, the sensitivity to Se in fish and aquatic birds varies greatly among species, while generally mammals are less sensitive to excess than egg-layers (Chapman et al., 2010).

Deficiency of Se in humans can cause increased incidence of cancer, cardiomyopathy, osteoarthropathy, reduction in male fertility, liver dysfunction, mood disorders, skeletal muscle disorders, impaired thyroid hormone metabolism, impaired immune function, and progression of HIV infection and morality (Chariot & Begnani, 2003; Bella et al., 2010). In terrestrial animals, such as sheep, pigs, or moose, Se deficiency can cause white muscle disease, mulberry heart disease, denaturing of globin subunits of hemoglobin, birth defects, or cause population declines due to reduced overall fitness (Chapman et al., 2010; Flueck et al., 2012). However, due to the complex nature of Se, animal requirements of Se depend on life stage, levels of pollution, and other factors that cause oxidative stress (Flueck et al., 2012).

Selenium's Effects on Mercury

It has been known since 1967 that Se has an antagonistic effect on Hg, with many studies supporting that an increase in Se concentrations correlates with a decrease in MeHg in tissue (Belzile et al., 2006; Khan & Wang, 2009; Dang & Wang, 2011). The toxic effects of exposure

to Hg in the presence of Se has also been observed to depend upon an organism's tissue Se to Hg molar ratio of 1:1, with more hazardous effects of Hg resulting when Hg is in excess of tissue Se (Chen & Belzile & Gunn, 2001; Khan & Wang, 2009; Sørmo et al., 2011). However, the mechanisms behind the interactions between Se and Hg, especially in regards to bioaccumulation, are complex and poorly understood, and are greatly dependent on the speciation of Se and Hg, the concentration of Se, and the species of the animal (Dang & Wang, 2011).

What is known, is that when an organism undergoes stress from metal exposure, such as Hg, a number of cellular proteins are activated to cope with the metal, including a protein called metallothionein (MT) (Sørmo et al., 2011). MT binds to unwanted metals, and this process triggers the production of more MT. When the Se ratio is greater than Hg, the production of MT is significantly reduced, indicating the stress caused by the Hg is decreased due to the protective effect of Se (Sørmo et al., 2011). However, this effect is dependent upon the relative concentrations of Hg and Se, the bioavailabilities of each element, and the sensitivity of the animal (Khan & Wang, 2009). There are a number of hypotheses of the potential pathways in which Se decreases the assimilation of Hg, or increases the elimination, or both, such as: MeHg-Se compound formation, Se aiding the demethylation of MeHg, inorganic Hg-Se compound formation, inorganic Hg redistribution in the presence of Se, and Se inhibition of methyl radicals from MeHg (Khan & Wang, 2009; Dang & Wang, 2011; Sørmo et al., 2011). Further studies are required to fully understand the interactions between Se and Hg, regardless of the known beneficial antagonistic effect of Se on Hg.

Study Lakes

Shortts Lake (SH) and Morris Lake (MO) in central Nova Scotia were sampled. Shortts Lake (45.2191, -63.3181) is located in Colchester County, while Morris Lake (44.6515, -63.4973) is located in Halifax County (Table 1). Shortts has a surface area of approximately 178.1 ha, a volume of 9228157 m^3, and a maximum depth of 14 m, while Morris is 130 ha, with a volume of 3693150 m^3, and maximum depth of 12.8 m (Table 1). Bathymetry maps can be found for Shortts, and Morris Lake (Figure 2). Both of these lakes had a pH slightly alkaline, with Shortts having a pH of 7.37, and Morris having a pH of 7.31 (Table 2).

The Shortts Lake shoreline was widely covered in a large quantity of aquatic macrophytes. An important potential contaminant source, the Lafarge Brookfield cement factory is approximately 100 m from Shortts lake. Morris Lake is located within the city of Dartmouth, and hence is surrounded by highly developed and populated areas; nearby is a park complete with a playground and a walking trail. Despite the high human traffic, the shoreline was covered in a large quantity of aquatic macrophytes as well. It should also be noted, that in addition to being located within Dartmouth, Morris Lake is located approximately 3 km away from the Imperial Oil refinery, a regionally important contaminant source.

From a geological standpoint, both the lakes are located within the Meguma Terrane, however, the geology differs greatly between the two lakes; Morris Lake is within of the Meguma Group, characterized by slates, schists, iron-manganese nodules, and sandstone turbidites, while Shortts Lake is within the Windsor Group (Figure 3), characterized by limestones and evaporates such as gypsum, limestone, dolostone, and anhydrite (Keppie, 2000). The Morris Lake geology can further be classified as falling directly within the sand-dominated

Goldenville Formation, which – reflective of its name – is a formation rich with gold deposits and the associated As rich mineral arsenopyrite (Ryan & Smith, 1998; Dummer et al., 2015). Consequently, the Goldenville Formation area in which Morris Lake is located has recorded arsenic levels of approximately 11-18 ppm in river sediments (Figure 4). In contrast, Shortts Lake has approximately 0-2.5 ppm arsenic levels in stream sediments (Figure 4).

The fish species from each lakes are similar with gaspereau (or alewife) *Alosa pseudoharengus* (GA), yellow perch *Perca flavescens* (YP), smallmouth bass *Micropterus dolomieu* (SB), chain pickerel *Esox niger* (CP), white sucker *Catostomus commersoni* (WS), white perch *Morone americana* (WP) and American eels *Anguilla rostrata* (AE) sampled in both. Shortts also had brown bullheads *Ameiurus nebulosus* (BB) in our sample set as well. Both smallmouth bass, and chain pickerel are invasive species to Nova Scotia, and are popular sport fish (Halfyard, 2008).

Fish Species of Interest

Smallmouth bass (SB) *Micropterus dolomieu* originally ranged in the Eastern United States to southeastern Canada, although are not native to Nova Scotia (Sternberg, 2001). SB were originally introduced to Nova Scotia as a sport fish, and are currently known to be in 188 lakes/rivers of Nova Scotia (Department of Fisheries and Oceans [DFO], 2009). Once introduced to a water body, SB can quickly outcompete other fish species, and reduce fish diversity (DFO, 2009), placing them in the category of invasive species. SB are greenish to bronze in color and can grow up to approximately 5 kg, and are opportunistic feeders known to eat invertebrates, crayfish, frogs, and other fish (Sternberg, 2001).

Chain pickerel (CP) *Esox niger*'s historical habitat ranged throughout the Midwest, Northwest and South of the United States (Stanberg, 2005). However, similar to SB, CP were introduced to Nova Scotia as a sport fish but then rapidly spread; they are aggressive opportunistic feeders that are known to eat invertebrates, frogs, crayfish, other fish, and even mice (Stanberg, 2005). CP are part of the pike family, can grow up to approximately 4 kg, and have a greenish to bronze coloration with a dark chain-link pattern (Stanberg, 2005).

White perch (WP) *Morone americana* is a native species to Nova Scotia and can be found throughout fresh water bodies in the province, and are known to be semi-anadromous (Livingstone, 1951). They can grow up to approximately 0.5 kg, and are silvery-white in colour with a darker shade along the top. WP are often opportunistic feeders which are known to have ranged diets from plankton, to invertebrates, to other fish (Zuerlein, 1981; Stanley & Danie, 1983; Parrish & Margraf, 1990).

Gaspereau (GA) *Alosa pseudoharengus* are another native species to Nova Scotia, and are called alewife in other regions of eastern North America. GA differ from the other species of interest as they are fully anadromous, plankton-feeding, and spend most of their adult life at sea (Bowden, 2014). They have a distinctive keel-like belly, with saw-tooth projecting scales along its belly. And they primarily feed upon plankton, but are also known to eat invertebrates and occasionally small fish. GA spawn in freshwater, and an individual could potentially complete five or more spawning migration throughout their 10-year lifespan (Bowden, 2014).

Objective

The purpose of this study was to analyze the trends in mercury, arsenic, and selenium in fish muscle tissue, with a focus on the species gaspereau alewife (GA), smallmouth bass (SB),

chain pickerel (CP), and white perch (WP), between a lake with low arsenic sediment and groundwater levels (Shortts Lake), and one with high levels (Morris Lake).

Methods

Field Sampling

Fish were collected in collaboration with the Nova Scotia Department of Fisheries and Aquaculture at Morris Lake on the afternoon of June 17, 2014, and at Shortts Lake on the morning of June 25, 2014. Collection used multi- and single panel monofilament gill nets (with 1", 1.5", and 2" mesh size), which were placed the overnight, and double and single wing fyke nets, and metal fish traps, which were set either the night before or four to eight hours previously.

To ensure cleanliness, previous in laboratory preparation of equipment included: pre-cutting of aluminum foil sheets, cleaning cutting boards with a 20% Nitric Acid rinse, and cleaning knives with soapy water. Both boards and knives were rinsed three times with type 3 reverse osmosis (RO) water, followed by three rinses of type 1 Ultrapure RO water. While in the field, a clean space was prepared for fish processing by covering all work surfaces, which consisted of three coolers at Morris Lake and an area on a cement boat dock at Shortts Lake, in clean unused plastic garbage bags.

To minimize cross-contamination between individual fish while processing in the field, the collected fish were firstly arranged by species, starting with species known to have lowest trophic level (e.g. typically associated with lower mercury concentrations) to highest-trophic level piscivorous species. Finally, American eels (AE) samples were processed last; due to the oiliness of muscle tissues making it difficult to clean knives and cutting boards between fish. Each species was then processed by size from smallest to largest.

Fish were then weighed to the nearest 0.1 gram, and measured from nose to tail fin tips to the nearest 0.1 centimetre with the use of a scale and fish measuring board respectively. Fillets of muscle tissue were then sliced from each side of the fish and wrapped in aluminum with scales to aluminum, while the tissue of the two fillets were in contact with each other. This was to increase the layers between the tissue and potential contamination. At this time, fish were also sexed, and stomach contents were examined and, if invertebrates were still fully intact or if contents required further identification, collected in a sterilized borosiliciate 20 mL scintillation vial. Between each fish processed the scale, boards, and knives were wiped clean with paper towel and RO water. Once snugly and completely wrapped in aluminum, the samples were individually sealed in a sterilized whirl-pak – which was labelled with the appropriate sample ID – and placed on ice until relocated to the laboratory freezer.

Sample Preparation

Clean laboratory procedures were followed to reduce the contamination of any samples in the laboratory. This includes the cleaning of the entire labs from the top of the shelves, as well as the inside cabinets, to the floor to remove any dirt or dust before any samples were processed. Clean, closed-toed laboratory shoes were worn to maintain the cleanliness of the lab and reduce outside contamination. Latex gloves were also worn whenever appropriate, such as handling samples or working with acid.

All non-metal instruments and borosiliciate 20-mL scintillation vials used to process samples were bathed in a solution 20% nitric acid and 80% ultrapure RO water for eight to 24 hours depending on the previous usage of the instrument (e.g. borosiliciate 20-mL scintillation

vials were brand new and therefore received at least eight hours, while petri dishes used to dry samples were acid washed for 24 hours). These instruments were then soaked in a bath of 100% ultrapure for at least six hours, which then - following removal -were rinsed three times with RO water and three times with ultrapure water. Glassware was dried in an oven reserved for drying, while other instruments were air dried on clean paper towel and covered by kimwipes. Metal and ceramic instruments, including knives and forceps, and cutting boards used were rinsed between each sample use in a solution 20% nitric acid and 80% ultrapure, rinsed in 100% ultrapure and dried using ultra-trace quality polyester knit cloths.

Similar to the field processing order pattern to reduce carryover of mercury, fish were processed by lakes; within lakes, they were processed by species of lowest known trophic level to highest, and within species, smallest specimens to largest. All AE samples were processed last due to oiliness of muscle tissue.

Fish muscle tissue samples were firstly slightly de-thawed, and then a proportion of muscle (depending on amount of sample) was removed from each fillet, avoiding all skin and scales. These sections were then diced, placed in a glass petri dish and dried in an oven between the temperatures of 50°C to 57°C for approximately 24 hours. Once dried, bones were removed, and the tissue was ground with a glass mortar and pestle (which was cleaned with 20% nitric acid, 80% ultrapure solution between each sample) until pieces were about the size of coarse sugar, and then placed into appropriately labelled 2.0 mL trace-metal quality micro-centrifuge tubes, with any excess sample placed into acid washed borosiliciate 20-mL scintillation vials.

Samples to be tested for Hg were ground into a consistent and fine powder with the use of a Retsch MM400 mixer mill and two 5-mm ultra-trace-quality zirconium oxide balls per a

micro-centrifuge tube; samples to be tested for stable isotope analysis, As or Se were ground with two 5-mm stainless steel surgical grade balls per a micro-centrifuge tube. Samples were run at 30.0 hertz per second for three to seven minutes or until all tissue was a consistent and fine powder. Zirconium oxide and stainless steel surgical grade balls were removed after grinding was completed, and washed using soapy water, rinsed with tap water, then RO water, and finally ultrapure water, and dried. This was done between each use.

Ground samples were then either readied for shipment to third party laboratories for the completion SIA, As and Se analysis, or placed in a desiccators with anhydrous "Drierite" brand desiccant crystals with ≥98% $CaSO_4$ and <2% $CoCl_2$ for in-house mercury analysis.

Analysis of Samples

In-house mercury analysis was completed with the use of the DMA-80 (Direct Mercury Analyzer). The DMA-80 contains 40 slots, that each holds a single quartz "boat" sample container, which makes up a single "run". Each quartz boat contained approximately 0.01 mg of sample or quality control material for analysis via combustion in the DMA. To ensure quality control and quality assurance, each run begun with standards, blanks, replicate samples, and certified reference materials (CRMs), and ended with two blanks. The CRMs used were as follows: lobster hepatopancreas for trace metals (TORT-2), dogfish protein for trace metals (DORM-3), and dogfish liver for trace metals (DOLT-4). The in-house replicates were two samples form Morris Lake and two from Shortts Lake. During the runs, blanks were placed between each grouping of fish species, and CRMs, and duplicate samples were re-analyzed approximately every 20 samples to monitor any possible fluctuations of the system. Each lake

was also analyzed separately, on separate days, to ensure the reduction of cross contamination. It should be noted that trace metal protocols were also observed throughout all laboratory processing and analyses.

Statistical Analysis

The data was then analyzed using SigmaPlot and Microsoft (MS) Excel. The correlations between THg versus TAs, THg versus TSe, and THg versus length, were all plotted with the use of SigmaPlot. For data that did show correlations, a linear regression was taken for each lake to determine the change in THg levels with increasing fish size. The regression produce the trend line equation to determine slope, and the r^2 value to establish the accuracy of the trend line fit to the data points. Averages and standard deviations for THg, TAs, and TSe, were determined and graphed using MS Excel. The values were compared between lakes and species of interest to determine if any trends were significant.

Results

The first notable trend was the positive correlation between THg concentrations and the size - determined by length - of fish (Figure 5). It was also determined that Shortts Lake had elevated THg concentrations compared to Morris Lake (Figure 5, Figure 6). However, while this trend was seen in the average THg concentrations of every species, it was only a significant difference in CP, YP, WP, WS, and AE (Figure 6). Regardless of the lake sampled, the average THg concentrations in all fish species of interest exceeded the World Health Organization (WHO) daily tolerable intake (based off the average weight of a Canadian person) of 0.0177 mg/kg (WHO, 2010; McMohen et al., 2012). Shortts Lake's species of interests were found to have an average THg concentration of 0.42±0.01 mg/kg for GA, 0.93±0.44 mg/kg for SB, and 0.69±0.50 mg/kg for CP (Table 3). Morris Lake's average THg concentrations were 0.33±0.10 mg/kg for GA, 0.74±0.33 mg/kg for SB, 0.35±0.21 mg/kg for CP, and 0.85±0.76 mg/kg for WP (Table 3). These values range from Shortts Lake SB with the greatest amount over the WHO (2010) daily tolerable intake at a difference of (Δ) +0.92 mg/kg, to Morris Lake GA with the smallest amount over at Δ +0.30 mg/kg (Table 4).

When the THg levels were analyzed with TAs and TSe levels, it was determined that there is no relationship in Shortts Lake nor Morris Lake between THg versus TAs levels, and THg versus TSe levels (Figure 7). However, both lakes had significantly elevated average TAs concentrations in GA when compared to the other species of interest (Figure 8). When the average TAs, and average TSe is compared between the same species of the two lakes, there is no significant difference in concentrations, yet some trends are suggested by the data. In general, Morris Lake had higher levels of TSe than Shortts Lake, while TAs concentrations appear to be

more species dependent rather than lake dependent, as the GA in Morris had higher levels than Shortts, but the CP had less, and the SB had nearly the same.

TAs levels in almost all species of interest were found to be above the WHO (2010) daily tolerable intake level - based off the average weight of a Canadian - of 0.233 mg/kg, with the exception of one WP sample (WHO, 2010; McMohen, 2012). Shortts Lake species of interests were found to have an average TAs concentration of 4.1±0.31 mg/kg for GA, 0.40±0.0060 mg/kg for SB, and 2.9±6.0 mg/kg for CP (Table 3). Morris Lake average TAs concentrations were 7.1±3.9 mg/kg for GA, 0.42±0.20 mg/kg for SB, 0.96±1.36 mg/kg for CP, and 0.22±0.063 mg/kg for WP (Table 3). These values range from Morris Lake GA with the greatest amount over the WHO daily tolerable intake at a Δ +6.9 mg/kg, to Morris Lake WP with a Δ -0.016 mg/kg (Table 4).

TSe levels in all species of interest were found to be above the WHO (2010) daily tolerable intake level of 0.31 mg/kg (WHO, 2010; McMohen, 2012). Shortts Lake species of interest were found to have an average TSe concentration of 1.4±0.13 mg/kg for GA, 1.3±0.12 mg/kg for SB, and 0.91±0.18 mg/kg for CP (Table 3). Morris Lake's average TSe concentrations were 1.4±0.31 mg/kg for GA, 1.6±0.27 mg/kg for SB, 1.3±0.11 mg/kg for CP, and 2.8±0.36 mg/kg for WP (Table 3). These values range from Morris Lake WP with the greatest amount above the WHO daily tolerable intake at Δ +2.5 mg/kg, to Shortts Lake CP with the smallest above at Δ +0.96 mg/kg (Table 4).

Discussion

There were no trends between TAs versus THg, and TSe versus THg concentrations in fish tissues (Figure 8). While this is not surprising in regards to TAs versus THg, as previous studies have not shown the two metals to significantly interact, it was somewhat expected that the TSe would affect THg due to the metals' known antagonistic interaction (Chen et al., 2001). It was also determined that within and across the two lakes TSe concentrations had no significant differences between the species of interest (Figure 7). However, the interaction between Hg and Se is complex, and previous studies indicate that different Se species and concentrations have variable effects on Hg assimilation in fish (Chen et al., 2001; Dang & Wang, 2011). Therefore, the lack of a relationship shown in the results may indicate that the Se is either not in an antagonistic species, or is not present in sufficient concentrations (or both), to significantly interact with the Hg in these lakes. However, further research would be required to determine this.

One significant trend was shown is the elevation of THg levels in Shortts Lake compared to Morris Lake (Figure 5). The higher Hg levels in Shortts Lake could be potentially due to the close proximity of the Lafarge Brookfield cement factory, which has reported annual emissions of 4.3 kg of Hg (Environment Canada, 2013b). Morris Lake's closest industry Hg-emitting source is the Dartmouth Imperial Oil refinery 3 km away, which reported annual emissions of 1.8 kg of Hg (Environment Canada 2013c). While the general trend of Shortts higher fish Hg levels than Morris is clear, when the average THg levels of each species is compared between each lake, only a number of species (CP, YP, WP, WS, and AE) have a significant difference between the two lakes (Figure 6). This could be due to a limited sample size, and with a larger

number of samples of each species from more lakes along a transect from the potential emission sources, a more conclusive trend could be revealed.

Perhaps a more concerning trend is that despite Morris fish having lower THg concentrations than Shortts fish, in both lakes all fish samples were consistently over the WHO (2010) daily tolerable intake for Hg of 0.0177 mg/kg (WHO, 2010). In Shortts Lake, the highest sample was elevated by a factor of approximately 56, while in Morris Lake the highest sample was elevated by a factor of approximately 19. This demonstrates that Hg is still a contaminant of concern in Nova Scotia, regardless of the lake or fish species.

Another surprising trend was the significantly elevated concentrations of TAs in GA from both lakes when compared to other species of interest. GA from Shortts Lake had approximately 1.2 times more TAs than other fish from Shortts, while GA from Morris had approximately 4 times more TAs than other Morris Lake fish. GA are different from the other three resident species, in that they have an anadromous life cycle linking marine and freshwater ecosystems (Bowden, 2014). However, previous studies have found that freshwater aquatic species are expected to be exposed to higher As concentration compared to marine species due to atmospheric deposition and direct input from geological and anthropogenic sources, such as mine tailings (Bowden, 2014). Therefore, it is possible that these significantly elevated levels are due to the As bioaccumulating mechanism or the manner As is stored by GA, rather than solely extraneous exposure sources. However, this clearly indicates that arsenic in GA needs to be further studied to understand the anomalously elevated TAs concentrations in this species relative to other fish species.

TAs lacked any other clear patterns between lakes and species, as no one lake had consistently higher average TAs levels in the species of interests than the other. As previously stated, TAs concentrations appeared to be more species-dependent rather than lake-dependent, since the GA in Morris had higher levels than Shortts, but the Morris CP had lower concentrations while SB had nearly the same TAs concentrations in both lakes. This indicates that the As in sediment and groundwater likely has had no significant link to bioaccumulation of As in the fish species of interest. Therefore, the elevated levels above the WHO daily tolerable intake are potentially due to atmospheric deposition caused by the same industrial sources that emitted Hg: the Lafarge Brookfield cement plant, and the Dartmouth Imperial Oil refinery and / or due to inherent biogeochemistry of each lake affecting TAs bioavailability to the biota within each food web.

Conclusion

The complex and growing issue of mercury and arsenic contamination due to anthropogenic activities is still a concern in Nova Scotia lakes. The elevation of these two contaminants can result in cascading effects as both bioaccumulate (mercury also biomagnifies) through the food chain, therefore have the ability to impact all trophic levels. While these effects require further study, as well as selenium's potential role to mitigate mercury impacts on biota, both are important concepts for the future.

The continued study of the interactions between geology, hydrology, and biota is also important for the future understanding of Nova Scotia's environmental and human health. Gaining further knowledge in these interactions could aid in the refinement of current policies and perhaps the development of new policies that could mitigate or reduce the anthropogenic release of mercury and arsenic, and hence ensure that levels remain safe for both the human citizens of Nova Scotia, as well as the wildlife occupants of its natural environment.

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Figures

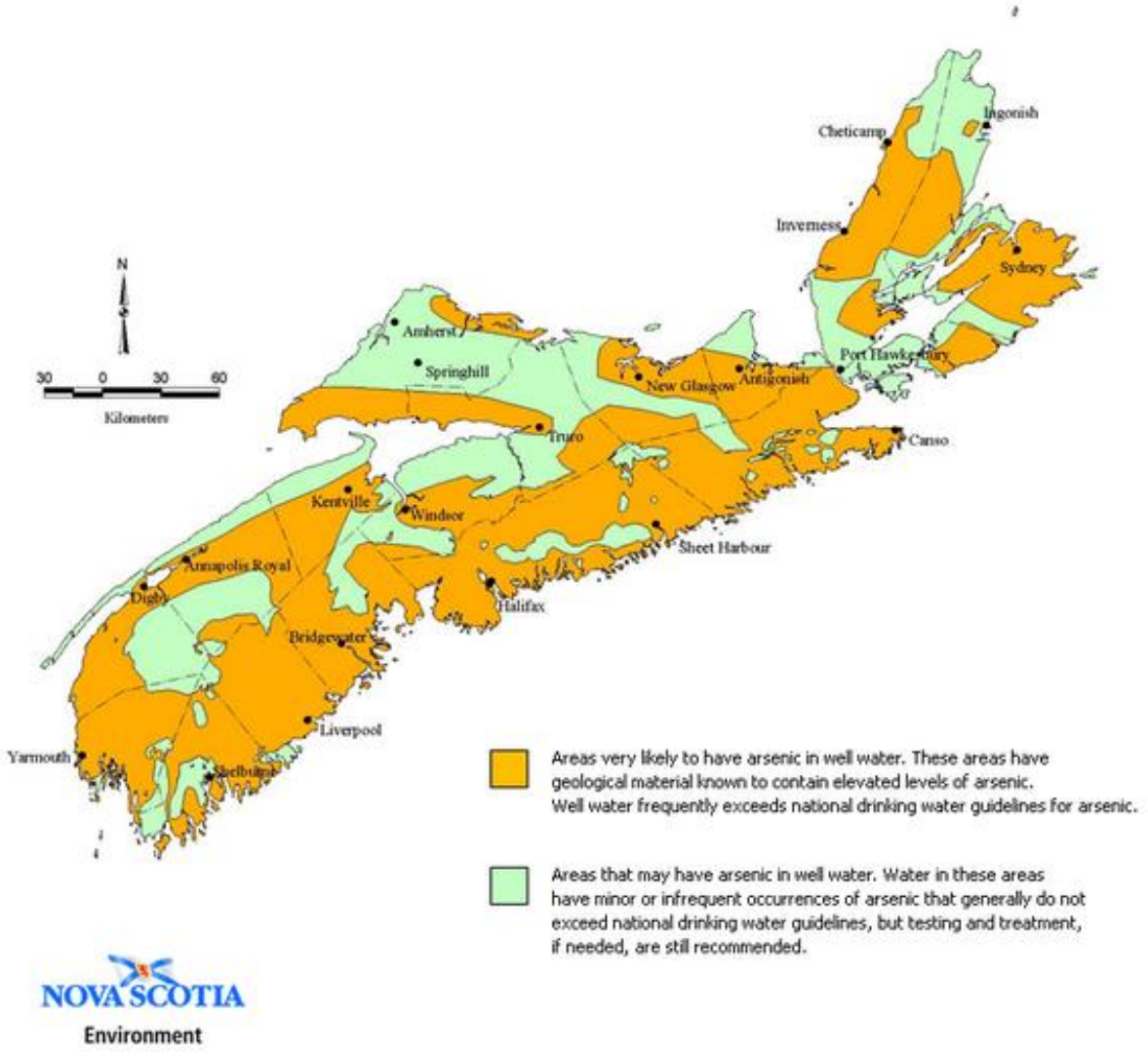


Figure 1: Areas with high frequency of elevated arsenic in groundwater in Nova Scotia; orange areas correspond with Meguma Terrane (Nova Scotia Department of Environment and Labour, 2008).

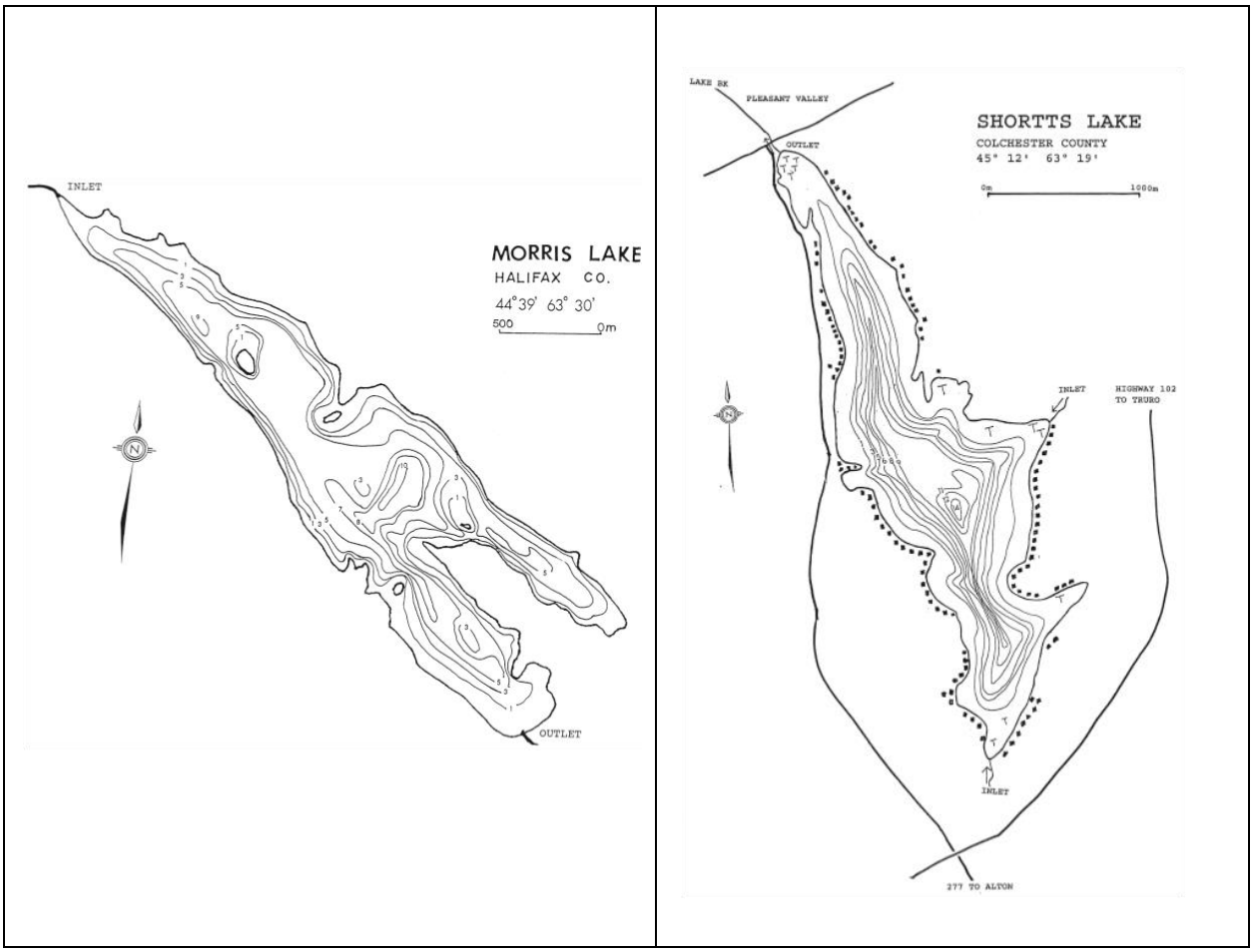


Figure 2: Bathymetric maps for Morris Lake and Shortts Lake, from the Nova Scotia Department of Fisheries and Aquaculture. Limnological data can be found in Table 2.

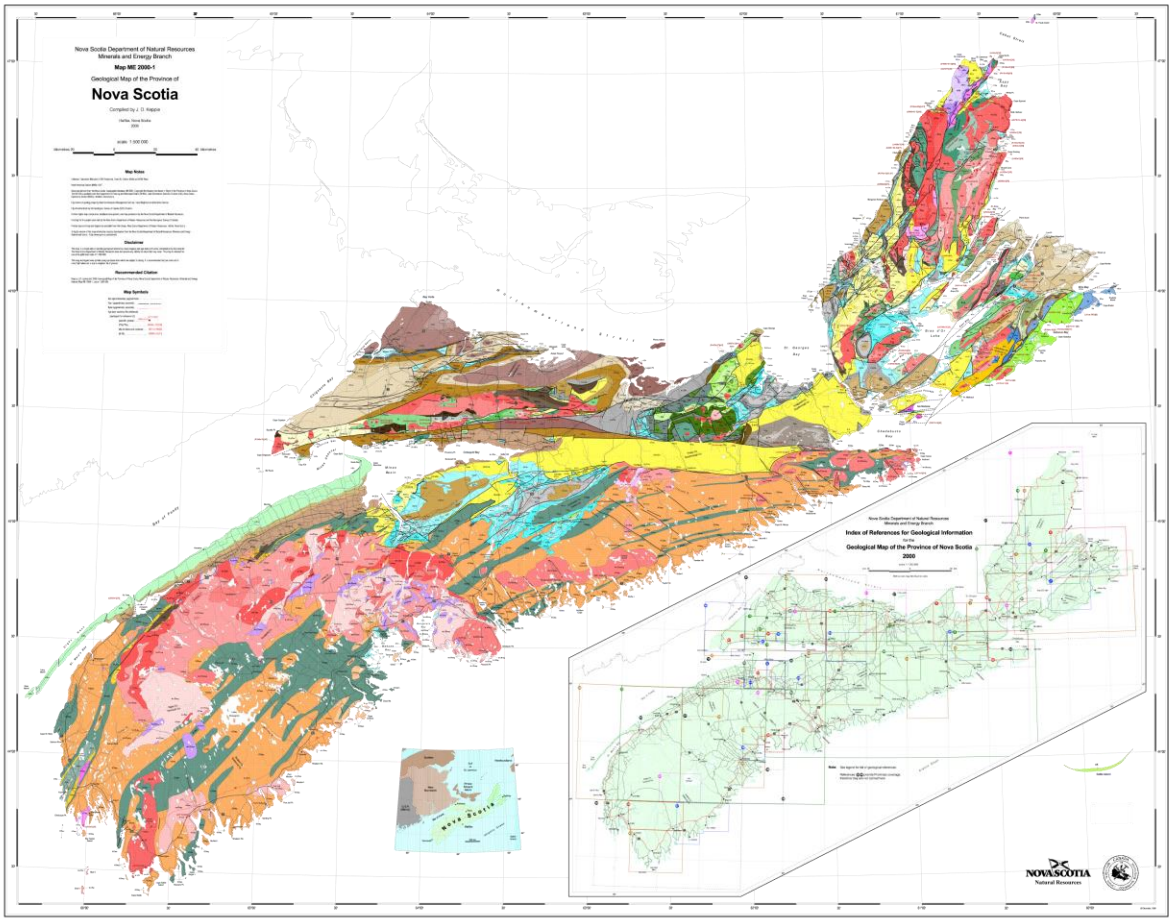
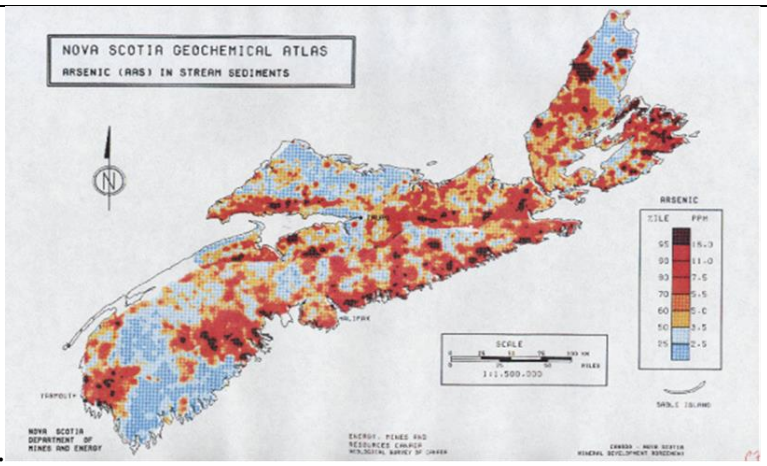
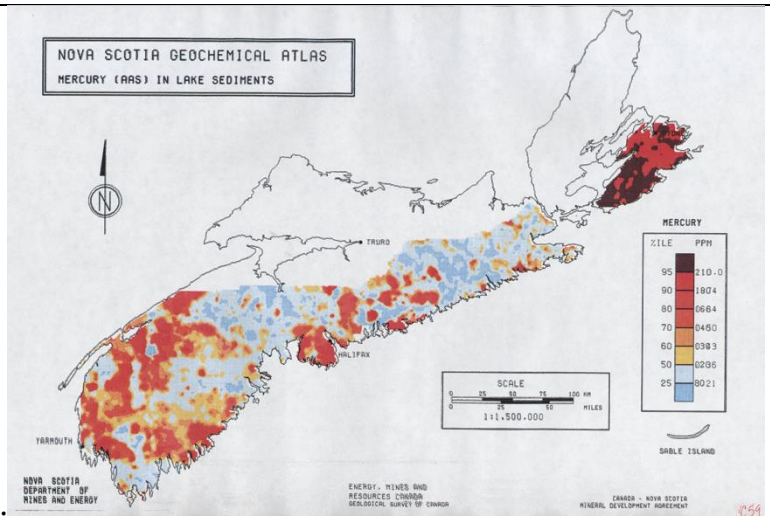


Figure 3: Detailed map of geological formations of Nova Scotia. Shortts Lake is located in the Windsor Group (blue, yellow and gray), while Morris Lake is located in the Meguma Group (orange and green) (Keppie, 2000).

a.



b.



c.

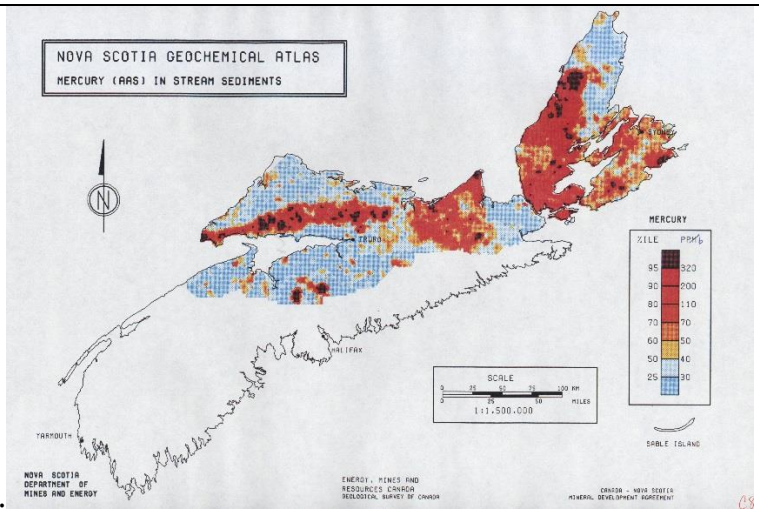


Figure 4: (a.) the arsenic concentrations of Nova Scotia stream sediments; (b.) the mercury concentrations of Nova Scotia lake sediments; (c.) the mercury concentrations of Nova Scotia stream sediments. No maps were available for selenium concentrations in stream or lake sediments, nor for the northern concentrations of mercury in lake sediments, nor the southern mercury concentrations of stream sediments (Nova Scotia Department of Natural Resources, 2013).

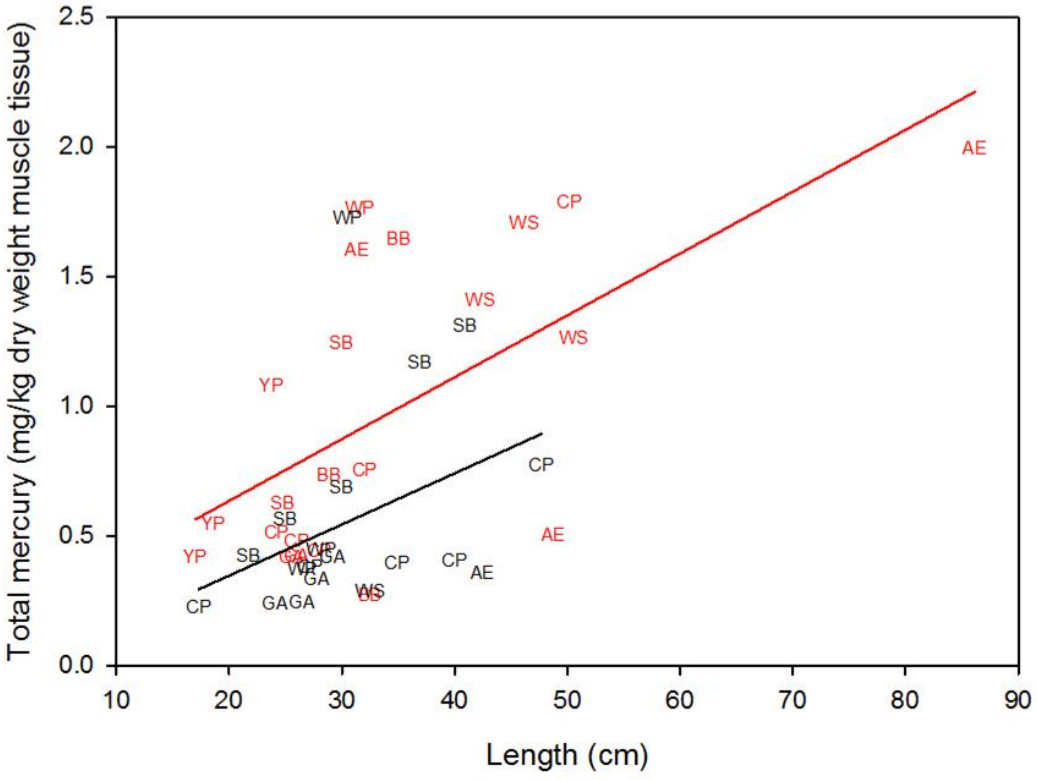


Figure 5: Total mercury concentrations (mg/kg) dry weight of fish muscle tissue versus length of fish (cm) of each fish sample from in Shortts Lake (red) and Morris Lake (black), with respective trend line. Individual sample species indicated by code. Samples collected during the 2014 field season.

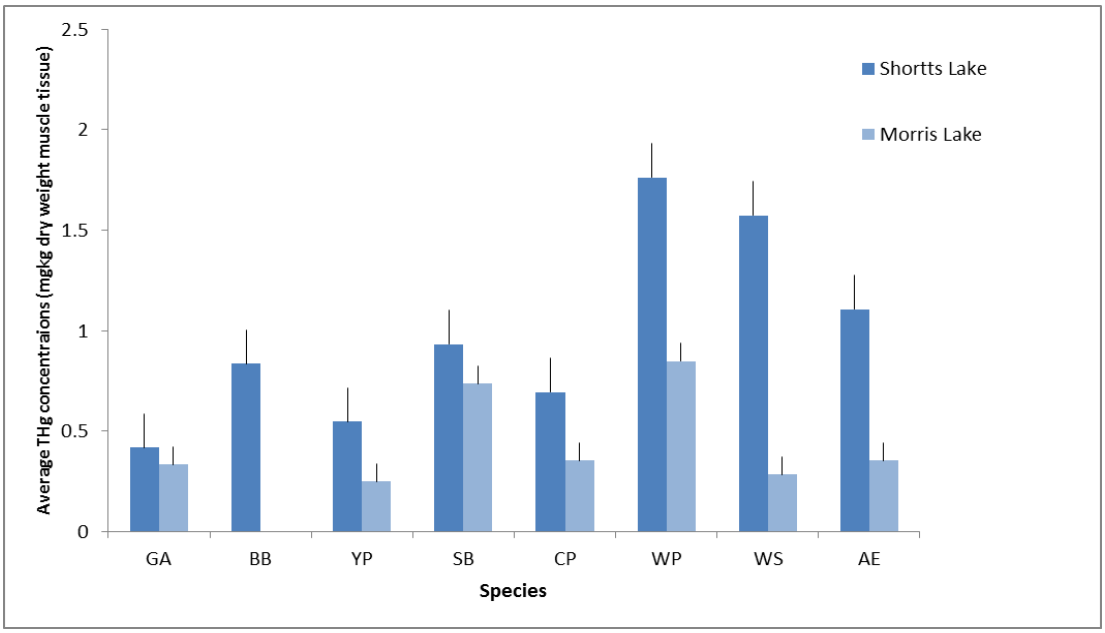


Figure 6: Average total mercury concentration (mg/kg) dry weight of fish muscle tissue versus species of Shortts Lake (dark blue) and Morris Lake (light blue), with standard deviation bars. Species indicated by code. Samples collected during the 2014 field season.

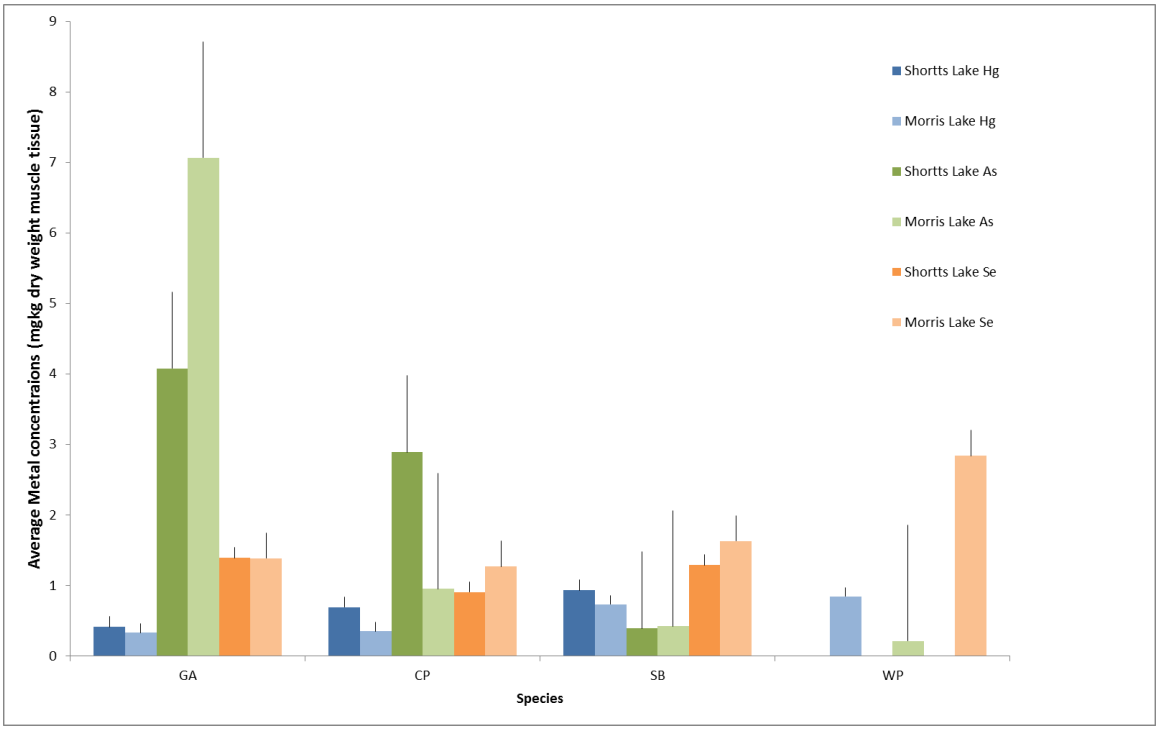


Figure 7: Average total mercury (blues), average total arsenic (greens), and average total selenium (oranges) concentrations (mg/kg) versus fish species of Shortts Lake (darker shade of each colour) and Morris Lake (light shade of each colour), with standard deviation bars. Samples collected during the 2014 field season.

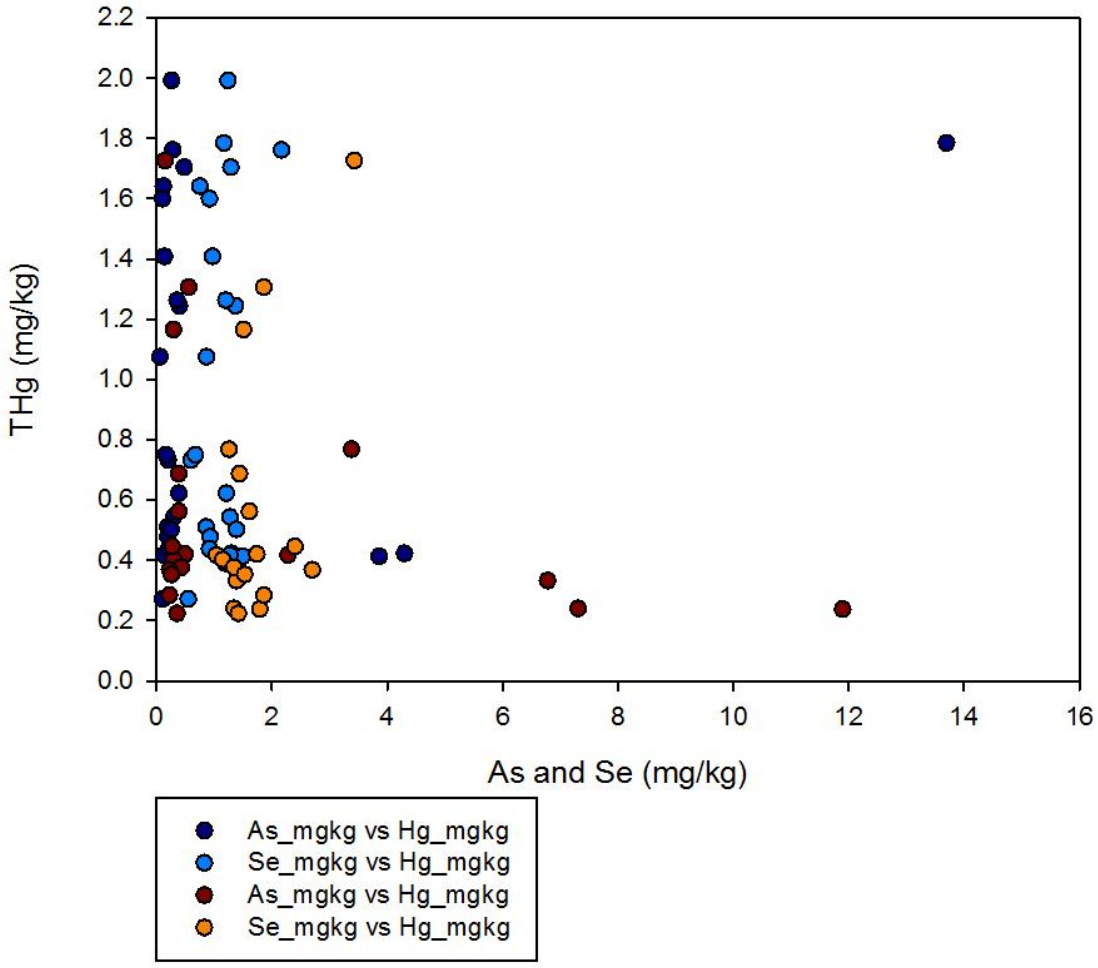


Figure 8: Total mercury concentrations (mg/kg) versus total arsenic and total selenium levels of all samples from Shortts Lake (black and blue), and Morris Lake (red and orange). Samples collected during the 2014 field season.

Tables

Table 1: Specifications of Shortts Lake and Morris Lake. Surface area data retrieved from Alexander et. al. (2006) and Nova Scotia Lake Survey Program (2014).

Date Sampled	Lake	Code	Latitude	Longitude	Max. Depth (m)	Surface Area (m^2)	Volume (m^3)
18-Jun-2014	Morris	MO	44.6515	-63.4973	12.8	130	3693150
26-Jun-2014	Shortts	SH	45.2191	-63.3181	14	178.1	9228157

Table 2: Water quality parameters collected by lake using a YSI 6600 multi-parameter water quality SONDE.

Parameter	Shortts	Morris
pH	7.6	7.6
Conductivity (µS/cm)	135.3	425
Total Organic Carbon (mg/L)	4.9	3.1
Alkalinity ($CaCO_3$ mg/L)	16.9	17.2
Sulfate (mg/L)	14.9	12.4
Secchi Depth (m)	3.7	4.1

Table 3: Average total mercury, arsenic and selenium concentrations (mg/kg) of gaspereau alewife, smallmouth bass, chain pickerel, and white perch in Shortts Lake and Morris Lake with standard deviations.

Sample Code	Number of Samples	Average THg (mg/kg)	Number of Samples	Average TAs (mg/kg)	Average TSe (mg/kg)
SHGA	2	0.418±0.007	2	4.080±0.311	1.400±0.134
MOGA	6	0.332±0.104	4	7.100±3.933	1.390±0.308
SHSB	2	0.933±0.440	2	0.395±0.006	1.290±0.116
MOSB	8	0.735±0.325	5	0.424±0.204	1.630±0.270
SHCP	7	0.693±0.496	5	2.900±6.042	0.910±0.179
MOCP	7	0.354±0.213	5	0.956±1.356	1.270±0.110
MOWP	3	0.846±0.763	3	0.217±0.063	2.840±0.356

Table 4: The average total mercury, arsenic and selenium concentrations (mg/kg) of the species of interest in Shortts Lake and Morris Lake, and the difference in value of each species from the WHO daily tolerable intake value (0.0177 mg/kg for mercury, 0.233 mg/kg for As, and 0.310 mg/kg for Se).

Sample Code	Average THg (mg/kg)	Δ[THg] fr. WHO guidelines	Average TAs (mg/kg)	Δ[TAs] fr. WHO guidelines	Average TSe (mg/kg)	Δ[TSe] fr. WHO guidelines
SHGA	0.418	+0.400	4.080	+3.847	1.400	+1.090
MOGA	0.332	+0.314	7.100	+6.867	1.390	+1.080
SHSB	0.933	+0.915	0.395	+0.162	1.290	+0.980
MOSB	0.735	+0.717	0.424	+0.191	1.630	+1.320
SHCP	0.693	+0.675	2.900	+2.667	0.910	+0.600
MOCP	0.354	+0.336	0.956	+0.723	1.270	+0.960
MOWP	0.846	+0.828	0.217	-0.0157	2.840	+2.530