Mercury Concentrations in Invertebrates From a Contaminated Wetland, Montague Gold Mines, Dartmouth, Nova Scotia

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Abstract

Mercury pollution has become a significant concern to environmentalists over the past several decades. With natural releases of mercury and increasing anthropogenic activities releasing larger quantities of mercury into the environment over the last several decades, harm to organisms and ecosystems is also on the rise. One anthropogenic activity that will be considered in this report is gold mining and its products' bioavailability, via mercury contaminated-tailing material, in aquatic invertebrates at a wetland located in the Montague Gold Mining District in Dartmouth, Nova Scotia. Sediment from the study wetland at Montague Mining District showed significantly elevated total mercury concentrations with all samples far above the Canadian Council of Ministers of the Environment freshwater sediment values. Focal invertebrates (Anisoptera, Zygoptera and Dolomedes) were collected from the study wetland as well and analyzed for total mercury content displaying significantly higher total mercury concentrations than invertebrates of the same sub-order and genus collected from a non-contaminated reference site. Additionally, Anisoptera total mercury levels far exceeded levels found at a similar study conducted at Kejimkujik National Park, indicating this site in particular is of high concern for mercury contamination, bioavailability and potential biomagnification through terrestrial and aquatic food chains. With respect to parental Hg transfer to young, our results suggest that Hg transfer from Dolomedes to their young within their egg sacs is likely. Due to the nature of the odonate and dolomedes lifecycles and their potential to spread contamination not only to terrestrial landscapes, but also to other aquatic habitats, , it is clear that the impacts go beyond the boundaries of the Old Stamp Mill wetland.

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Introduction

Mercury: Not Just a Regional Issue

Mercury pollution has become a monumental concern to scientists and environmentalists over the past several decades. The understanding and research of the complexity and speciation of mercury and its effects on the environment and various implications it has on animal health has been thoroughly studied due to this elevated concern. Estimates indicate that nearly 3000 tonnes of mercury is released annually (2005) into the atmosphere from naturally occurring processes, while nearly 2500 tonnes is released via anthropogenic activities (Pirrone & Mahaffey, 2005). Mercury pollution has progressively become realized as not only a concern of a regional scale, but a major global issue. With natural releases of mercury and increasing anthropogenic activities releasing larger quantities of mercury into the environment over the last several decades, harm to organisms and ecosystems is also on the rise.

Toxic Effects of Mercury and Its Implications on Aquatic Environments

Mercury is an element that occurs naturally in the environment via volcanism, natural erosion, vegetation, and forest fires (Jiang, 2013). It can also be released via anthropogenic disturbances such as combustion of coal, incineration of waste (Jiang, 2003) as well as mining (Gochfield, 2003). Elemental mercury (Hg^0) is capable of global and long-range transport via the atmosphere (Jiang, 2013). Hg^0, which is mainly found in the atmosphere, is capable of binding to water surfaces, but can also react with atmospheric oxidants to produce inorganic mercury (Hg^{2+}). Hg^{2+} can bind more easily to water and particles (Hsu-Kim, 2013). Once Hg^0 becomes oxidized to create Hg^{2+}, it is

highly soluble and readily absorbed by precipitation, which enters biological habitats (Gochfield, 2003). Methylmercury (MeHg) is created with Hg^{2+} acting as the substrate for the methylation process, which can have deleterious effects on the organisms exposed to it (Colombo, 2013).

Aquatic environments act as a major source of MeMg production. MeHg can be created when Hg^{2+} enters water systems and settles to the sediment at the bottom of the water to form mercuric sulfide (Gochfield, 2003). If conditions are anaerobic, methylation of mercuric sulfide can occur via methylating bacteria to create MeHg (Colombo, 2013).

MeHg is the most bioavailable (most readily taken up) and toxic form of mercury to organisms. In organisms like fish and humans, eating mercury-containing organisms allows for MeHg to easily pass through the gastrointestinal tract, become blood borne and subsequently reach vital organs causing physiological issues such as central nervous system impairment, reproductive impairment, overall poor health and even death (Gochfield, 2003).

Once in water systems and aquatic organisms, terrestrial transmission of MeHg via emergent insects and aquatic-feeding terrestrial predators such as bats and birds can occur (Little et al. 2015). Aquatic organisms can serve as bioindicators of contaminants and analyzing their tissues can reveal information on the types and bioavailability of contaminants that are present in the area. For insects with significant aquatic life cycles such as dragonfly, mayfly and damselfly nymphs, research has shown that mercury can bioaccumulate (continue to accumulate) in their tissues and can act as important vectors for transmission of mercury to other aquatic organisms and terrestrial organisms

(Buckland-Nicks et. al, 2013). Aquatic spiders have been shown to contain some of the highest concentrations of mercury compared to other invertebrates (Sullivan & Rodewald, 2012), particularly in areas with high concentrations of mercury in nearby sediments. Montague Gold Mines is an example of a highly mercury contaminated area, and is the selected site for this study. For a significant period of time in which the mine was in operation, gold was extracted involving amalgamation via the use of mercury. The by-products of this process produced tailing material, or mercury-containing crushed ore, which was disposed of either in water bodies or directly onto the land (Bates, 1987). This gold mining disposal process can act as an important source of mercury contamination in surrounding soils, sediment and water.

History of Gold Mining in Nova Scotia

There were 3 key periods in Nova Scotia when there were "gold rushes" in which people from all over North America traveled to the province to seek gold. After a number of unofficial discoveries made by farmers and laborers building roads across Nova Scotia between the 1830's and 1850's, the first gold rush was initiated in 1860 after a Musquodoboit farmer discovered a large amount of gold in a quartz boulder (Bates, 1987). Soon after the discovery, mining companies began new exploration and expansion and gold production increased drastically across Nova Scotia between 1860 and 1867. The first gold rush came to a slow decline over the next few years and by 1874 gold production had decreased greatly due to poor mining techniques and improper management (Bates, 1987). With improved techniques for extraction of gold, the use of explosives and improved management employed by mining companies, approximately

882 kilograms of gold were extracted during the second gold rush, which spanned between 1896 and 1903 (Bates, 1987). The third gold rush in Nova Scotia occurred between 1932 and 1942 and yielded approximately 4479 kilograms of gold (Bates, 1987). Although various methods and techniques were used to the extract gold during these times, one of the most environmentally damaging techniques used was the mercury amalgamation process. This technique was used primarily during the first gold rush; although it is still practiced today, and involved pouring liquid mercury over crushed ore in order to physically separate the ore from the gold (Bates, 1987). The gold would dissolve into the mercury creating an amalgam. The amalgam was then heated at high temperatures to evaporate the mercury, leaving behind the gold (Bates, 1987). This process resulted in significant amounts of mercury-contaminated crushed ore and increased mercury vapour emissions at the site. This crushed contaminated ore, called tailing material, was then disposed of onto the land or directly into water bodies (Bates, 1987). This practice contributed to polluting the environment in two ways: the evaporation of the mercury during the separation of the amalgam contributed to increased amounts of atmospheric mercury, while the unregulated disposal of tailing material contributed to severe contamination of the soil, sediment and water quality in the area. These mine tailings still threaten to have effects on the environment even over a century later.

Montague Gold Mines

Montague Gold Mines is located in Dartmouth (44°43’06”N, 63°30’48”W) off Montague Road. Montague Gold Mines was first discovered to contain gold in the 1800’s

and gold mines, also referred to as stamp mills, were operational within the area from the late 1800's to the early 1900's (Saunders, et. al., 2010). Lake Major, one of Halifax Water's regional watersheds, lies to the East of Montague Gold Mines, while Loon Lake and Lake Charles lie to the south and to the west, respectively. A slow moving brook (Mitchell Brook) connects Loon Lake and Lake Charles with several wetlands and tributaries feeding into this river. Forests predominantly made up of white spruce trees surround the area, while the forest floor is predominantly covered in moss. Areas with significant amounts of old tailing material lack vegetation and the ground is barren and covered in fine grey-brown, silty sediment (Figure 2). Given that many interconnected waterways and aquatic ecosystems lay within the district, aquatic contamination of mercury is of particular concern.

Wetlands as Significant Sources of Mercury Contamination and Dissolved Organic Matter

Canadian freshwater ecosystems are of critical importance to the health and biodiversity of aquatic organisms and are intimately interdependent on other ecosystems and habitats in addition to being of significant economical benefit to the import and export of major Canadian goods and products. The health and preservation of Canada's wetlands are imperative for the stability of freshwater ecosystems as they provide many important functions. Wetlands can be classified as any area of land either seasonally or permanently inundated with water (Province of Nova Scotia, 2011)

The preservation of wetlands has is an ongoing compelling concern. Urbanization, over-development, pollution and other anthropogenic activities can have detrimental

effects on these important aquatic assets. Wetlands have also been identified as important areas for the filtration of sediments and potentially toxic contaminants before they enter larger aquatic ecosystems such as lakes and oceans (Province of Nova Scotia, 2011). Studies show that wetlands contribute significantly to the concentrations and mobility of MeHg as they can act as potential sinks for various species of mercury accumulation (Selvendarin et al., 2008). Watershed activities and processes as well as the presence of other contaminants greatly influence the transport of mercury into wetlands and the potential speciation of MeHg, which is primarily facilitated by sulfate reducing bacteria (Selvendarin et al., 2008).

Dissolved organic matter (DOM) and particulate organic matter (POM) typically are concentrated in wetland sediment and waters. DOM constitutes a proportion of total organic material (TOM). Loss on ignition is a commonly used and relatively easy procedure to perform to determine the amount of TOM in sediment. To determine the amount of total organic carbon (TOC) in a sediment sample (the amount of organic carbon contained in the TOM of the sample), the sample must be dried at a low temperature to remove water and obtain a constant weight (Heiri, et al., 1999). Next the dried sediment sample is weighed and then placed in a muffle furnace at 550°C for 4 hours, to allow for combustion of organic matter to carbon dioxide and ash (Heiri, et al., 1999). The percentage of weight lost between the drying of the sediment and the combustion of the organic matter following 4 hours of exposure to 550°C has been shown to correlate strongly with the amount of organic carbon contained within the sediment sample (Heiri, et al., 1999).

Since total organic carbon of sediment consists of two components, dissolved organic carbon (DOC) and particulate organic carbon (POC) (U.S. EPA., 2001), an increase in TOC would result in increases of DOC and POC of sediment or sediment pore water. DOC is the carbon content of dissolved organic matter (DOM) (Hopkinson, C.S. & Vallino, J.J., 2005), thus greater amounts of DOC in sediment or sediment pore water would correlate to greater amounts of DOM. Therefore, it can be inferred that increased loss on ignition (LOI) values correlate to increased TOC and ultimately, increased DOC and DOM values. DOM can be found in the sediment and waters of aquatic ecosystems and is heavily concentrated in wetlands. DOM is known to have a strong binding affinity for both inorganic mercury and MeHg due to its reduced sulfur-containing functional groups of amino acids and proteins (Hill et al., 2009). This increased binding affinity of inorganic mercury and MeHg to DOM aid in the facilitation of mercuric transport (Hill et al., 2009). DOM may also provide nutrients for bacterial communities in the absence of sulfate, which can stimulate microbial growth, thereby increasing methylation rates (Ravichandran, 2004).

DOM and POM are known to have a strong binding affinity for both Hg^{2+} and MeHg due to their reduced sulfur-containing functional groups of amino acids and proteins (Hill et al., 2009). This increased binding affinity of Hg^{2+} and MeHg to many DOM and POM compounds aids in the facilitation of mercuric transport (Hill et al., 2009). Hill et al (2009) found that DOM with low molecular weights were most associated with MeHg in wetlands in a survey of 20 freshwater sites across Ontario and Quebec. This has implications for increased MeHg entering food webs through uptake in invertebrates (Hill et al., 2009). Conversely, other studies suggest that high organic

matter can decrease methylation of Hg^{2+} by outcompeting sulfide for increased binding affinity to Hg^{2+}. When bound to DOM, inorganic mercury is unable to cross the cell membranes of methylating bacteria due to its increased size (Ravichandran, 2004). MeHg also has strong binding affinity for DOM, with increasing DOM concentrations potentially causing increased MeHg-DOM binding, thereby limiting uptake of MeHg by other aquatic organisms (Dong, et al. 2009).

Although there are interactions between DOM and MeHg, exactly how DOM relates to the bioavailability of MeHg in organisms is unclear as many factors are involved in the transformation of mercury species. Such factors include; water pH, water temperature, available nutrients, and the chemical composition of both the water and sediment (Selvendiran et al., 2008). Wetlands have been identified as important areas for the filtration of sediments and potentially toxic contaminants (Province of Nova Scotia, 2011), and are also imperative for aquatic biodiversity and organisms such as dragonflies, damselflies and aquatic spiders, among other invertebrates. These organisms are important food sources for a variety terrestrial organisms and other vertebrate aquatic life (Province of Nova Scotia, 2011) and therefore have the potential to contribute to the biomagnification (increase in a substance as it moves up the food chain) of associated contaminants to higher tropic levels and terrestrial landscapes (Buckland-Nicks et. al, 2013).

Common Wetland Invertebrates collected from the Old Stamp mill Wetland

Dragonflies and damselflies belong to the insect order Odonata, with dragonflies in the sub-order Anisoptera and damselflies in the suborder Zygoptera. Dragonflies and

damselflies share a similar life cycle, with the egg phase underwater, followed by an underwater 1-2 year nymphal stage and lastly, the flying adult phase (Burns & Reitsammer, 2009). Odonante nymphs are typically predacious and opportunists with respects to feeding (Pritchard, 1964). They typically will feed on what is most abundant within the environment during that season and usually will not attack prey above a certain size. Other factors that contribute to their feeding include the prey's ability to escape quickly, the overall toughness of the exoskeleton and the prey's ability to be undetected to swiftness in movement and small size (Pritchard, 1964). One study conducted on dragonfly larvae feeding behaviour was focused on examining the fecal pellets of a variety of species of dragonfly larvae from several ponds in Northern Alberta (Pritchard, 1964). Results indicated that chironomid larvae were most popular among all species of dragonfly larvae diets, followed by small Crustacea, Zygoptera larvae and coleopteran larvae (Pritchard, 1964). Adult beetles, other dragonfly larvae and non-aquatic insects also consisted of a smaller portion of their diet. In general, dragonfly larvae feeding habits vary based on prey size, availability, speed and the capability of their labial pulps to pierce and break apart prey, which is often dependant on the toughness of prey's exterior (Pritchard, 1964).

The semi-aquatic fishing spider of the genus Dolomedes are known to prey on a wide variety of organisms ranging from aquatic and semi-aquatic invertebrates to vertebrates such as small fish and frogs (Zimmerman and Spence, 1989). Equipped with special sensory organs that allow them to detect surface water vibrations, Dolomedes are particularly predacious to several species of the aquatic Hemiptera organisms commonly known to live on or near water surfaces. They tend to be the most commonly preyed upon

organisms as they are typically the most abundant and reliable sources of food for Dolomedes (Zimmerman and Spence, 1989). In addition to their exceptional water surface hunting capabilities, Dolomedes are also capable of diving under water for extended periods of time in search of prey (Zimmerman and Spence, 1989). In addition to Hemiptera invertebrates, Odonates, particularly members of the suborder Anisoptera, are frequently preyed upon depending on seasonality and abundance (Zimmerman and Spence, 1989).

Objectives

The purpose of this project was to assess whether or not mercury was present in the sediment of the study wetland at Montague Gold Mines. Given that aquatic invertebrates can act as important indicators of mercury contamination and bioavailability, this research will also focus on whether or not mercury is bioaccumulating in a variety of aquatic invertebrates collected the study wetland in the Montague Gold Mining District (Figure 1).

Methods

The study site

The study wetland chosen for this study is located just outside of the main Montague Gold Fields and situated next to a tailing outfall from an old stamp mill thought to be established in the early 1800's. Due to its location outside of the Montague Gold Fields, we have informally named this site the "Old Stamp Mill" (OSM) wetlands, to be used for the purpose of this study to avoid confusion with other wetlands. Little data exists for this wetland site, however, some data is available on contaminant concentrations in soils up gradient of the OSM wetland area, all of which contain lower THg concentrations than those found in areas of the OSM wetland sediment (Parsons & Little, 2015; Chapman et al. 2015). Most of the OSM wetland area sediment is covered with waterlogged tailings upon which there are some growing plants. There is no data or maps detailing the extent of the tailing deposit in the wetland area. The OSM wetland is sparsely vegetated along the shoreline nearest the tailing outflow (Figure 3). Vegetation was more abundant and green in areas farthest from the shore and closest to the Mitchell Brook. Water levels are shallow, with only approximately 60 centimeters of water in areas closest to the shoreline, and approximately 90 to 105 centimeters at areas furthest from the shoreline, however after multiple visits between May and August of 2015, water levels seemed to fluctuate slightly.

The Reference Sites

2 reference sites (Ref. 1 & Ref. 2, respectively) were within relatively close proximately of each other and slightly upstream and across from the OSM wetland to ensure consistent reference site habitat and ecosystem variables as the OSM wetland. Vegetation was abundant and green within both reference sites, and water levels approximately 60 to 90 centimeters deeper than that of the OSM wetland.

Sampling

Sediment and invertebrate sampling was conducted on June 26th and July 3rd 2015 at the OSM wetland, and the two reference wetlands were sampled on July 13th 2015. Weather conditions during all sampling trips were warm and sunny. The water pH was recorded to be 6.3 at the OSM Wetland at the time of sampling. The water pH of the reference sites were not recorded.

The OSM wetland was divided into 4 quadrants, Quadrant 1 (Q1), Quadrant 2 (Q2), Quadrant (Q3), and Quadrant 4 (Q4). Each quadrant was roughly 15 meters by 15 meters, although due to the uneven geography of the wetland, this was difficult to keep precise (Figure 4).

Sticks were used at each point of each quadrant and placed upright with bright orange tape attached to the stick tops to create quadrant boundaries. Invertebrates were collected using dip nets. For invertebrate sampling from areas of sediment, dip nets were scraped across the top few centimeters of sediment layers 2 to 3 times and brought to the surface of the water for inspection. Dip nets were used to quickly disturb the base of any

patches of vegetation and then used to sweep and catch any invertebrates falling loose from the disturbed vegetation (Figure 5).

Acid-cleaned glass vials were labeled by quadrant prior to invertebrate collection. Invertebrates were picked out using cleaned plastic forceps and placed in the appropriately labeled glass vials corresponding to that quadrant. Vials were then filled with water from the wetland, sealed in clean-labeled plastic self-sealing bags and taken to shore where they were stored in a cooler with cold packs. At the same time, a representative invertebrate from each taxonomic group was placed in 70% ethanol for future species identification. This procedure was followed for collection of invertebrates from each quadrant and each reference site.

Collection of surface sediment was obtained using a small trowel with approximately 25 centimeters of sediment collected from the center of each quadrant. Sediment samples were not collected from reference sites. After each sediment sample had been collected, the spade was rinsed with wetland water. Sediment samples were placed in triple-bagged Ziploc bags, labeled and placed on cold-packs in a separate cooler that the invertebrates to prevent cross contamination.

Sample Storage and Preparation

Invertebrate and sediment samples were brought back to the lab immediately after collection. Sediment samples were stored in the fridge, while invertebrate samples were emptied of wetland water, rinsed with ultra pure 18 ohms ultra pure water and grouped according to physical similarities, characteristics and quadrant in acid-cleaned glass vials and placed in the freezer (Figure 6). While we collected multiple invertebrate taxa, for

this study, I focused on Anisoptera, Zygoptera and two groups of spiders (Aranae), however we expect these two spiders are the same species just different sizes. The data for other invertebrate groups are available upon request.

The ethanol-preserved Odonates were identified to genus, sometimes to species whenever possible (Merritt and Cummins, 1984; Clifford, 1991). In some cases, different species of the same genus were pooled together to ensure sufficient analytical mass for the mercury analyses, and those were labeled by that genus. The spiders represented a particular identification challenge, as we were not able to confirm to species. As a result, we will be sending representative specimens to an Araneae expert for confirmation. Pending confirmation, we are punitively labeling the two groups of spiders Dolomedes based on similarity to photographs of confirmed species in a published guidebook (Weber, 2003) and the overlap with known habitats and distribution maps. At the OSM wetland many species of spiders were found, but for this thesis, we included only two spider taxa each of which had very distinctive colouring and size, “Large Dolomedes” (approximately 17 mm, greenish-gray in colour with distinct white and blue stripes and spotting along the body) and “Small Dolomedes” (approximately 8 mm, greenish-gray in colour with distinct white and blue stripes and spotting along the body). We also analyzed egg sacs borne by two large Dolomedes separately.

Frozen sorted invertebrates were placed in a drying oven and heated at 50°C for 48 hours until dry. Once dried, invertebrate samples were removed from glass vials and placed in the Retsch mixing mill where they were ground into a fine powder. To prevent cross-contamination between invertebrates of different taxonomic groups, quadrants and reference sites, the mill stainless steel containers and grinding balls were cleaned in-

between each invertebrate grinding process with 10% Decon © pharmacological-grade detergent diluted in reverse osmosis (RO) water. All fat and tissue residues were removed. Once ground, invertebrates were transferred into sterile cryogenic vials and placed into desiccators to dry to consistent weight until analysis.

All sediment samples were removed from Ziploc bags, placed onto aluminum foil boats and dried in the oven at 50°C for 48 hours. Once dried, the sediment was ground into a fine powder by quadrant using the Retsch mixing mill. To prevent cross-contamination between quadrant samples, in-between each sediment grinding process, gloves and bench top lab paper were changed, and the stainless steel containers and grinding balls were decontaminated with diluted Decon© detergent as for the invertebrate samples.

Total Mercury Analysis

Trace-quality protocols were followed to ensure no cross contamination of samples occurred. The clean room was prepared to Class-1000 specifications, with all surfaces of the ultra-clean room (HEPA-filtered) wiped down with water and a gentle soap, followed by a Decon © detergent wash, tap water rinse and then an ultra pure water rinse. The use of latex gloves was employed for each sample and gloves were changed in-between the handling of each sample.

All samples were analyzed using the Milestone Direct Mercury Analyzer DMA 80.3 (DMA-80). Prior to each analysis run, blanks and a series of reference mercury standards (0ng, 5ng, 10ng and 20 ng) were prepared and run for accuracy. In addition, for each run, certified reference materials were run to ensure calibration was accurate.

Certified reference materials used for sediment runs were Dolt5, Mess3, and PAC2. Three samples from Quadrants 1, 2 and 4 and two samples from Quadrant 3 were run in nickel boats and an average of total Hg was calculated for each quadrant. After sediment analysis was completed, the DMA-80 was run through 3 cycles of complete 40-nickel boat burns to ensure any extra mercury remaining from the very elevated sediment samples was flushed out of the system and would not compromise future runs. After all boat burns were complete, all surfaces of the ultra-clean room were wiped down with water and a gentle soap, followed by a Decon © detergent wash, tap water rinse and then an ultra pure water rinse prior to any analysis of invertebrates to ensure no cross contamination of samples occurred.

Invertebrate samples were analyzed between January 23rd and February 4th 2016. Prior to each analysis run, blanks and a series of reference mercury standards (0ng, 5ng, 10ng and 20 ng) were prepared and run for accuracy. In addition, for each run, certified reference materials were run to ensure calibration was accurate. Certified reference materials used for invertebrate runs were NIST 2976, TORT3, and DORM4. Each sample was placed in an acid-washed, clean, dry quartz boat, weighed and recorded on the DMA-80 computer software. To prevent contamination carry-over, a blank quartz boat was placed in between invertebrate samples from differing quadrants and site locations.

Percent Loss on Ignition for Sediment Samples

In order to estimate total organic matter of sediment samples, % Loss on Ignition (% LOI) was used. Sediment samples from each quadrant were placed into separate aluminum foil boats and dried at 60°C for 36 hours to rid samples of any water content.

Once completely dried, three sediment sample replicates from each quadrant were removed from the oven and approximately 2 grams of each sediment sample were placed into clean, dry ceramic crucibles. The sediment-containing crucibles were then placed on the scale and the weight was recorded. A high temperature muffle furnace was set to 550°C. Upon reaching 550° each crucible was placed into the muffle furnace using heat resistant gloves and tongs for handling. Once placed into the furnace, the crucibles were heated at 550°C for exactly 4 hours. Once the 4-hour time period had elapsed, the crucibles were removed and subsequently weighed to determine weight lost between pre and post 550°C heating. Weight lost was converted into a percent lost of initial dry sediment weight to determine the percent of organic matter in each sediment sample (Rosenmeier & Abbott, 2005).

Data analyses

The data was analyzed using Microsoft Excel. Box plots were created to determine the difference in total Hg (THg) content in OSM wetland Anisoptera and combined reference site (RS) Anisoptera. A box plot was then created to determine the difference in total Hg content between OSM wetland Zygoptera and RS Zygoptera. Box plots were created to compare OSM wetland THg values between Anisoptera, Zygoptera and Dolomedes spiders (small and large). A table was created comparing the difference between THg in OSM wetland quadrant sediment samples and %LOI values for each quadrant. THg in sediments were also compared to %LOI values for their corresponding OSM wetland quadrants, while THg in Anisoptera, THg in Zygoptera , and THg in both

small and large Dolomedes spiders were compared to %LOI for their corresponding OSM wetland quadrant of collection.

Results

Concentrations of THg in Odonate species from the OSM wetland and RS were clearly different, with the OSM wetland Odonates being highly elevated in total Hg (THg). OSM wetland Zygoptera showed the most dramatic difference between THg concentrations when compared to those of the RS Zygoptera (Figure 7). Average THg concentrations in OSM wetland Zygoptera were 1.9±0.39 mg/kg, while average THg concentrations in RS Zygoptera were 0.17±0.13 mg/kg.

OSM wetland Anisoptera showed substantially higher total mercury content than those from the RS Anisoptera (Figure 8). Average THg concentrations in OSM Anisoptera was 1.4±0.24 mg/kg while average THg concentrations in RS Anisoptera was 0.24±0.02mg/kg.

When comparing THg levels in invertebrates within the OSM wetland, including Anisoptera, Zygoptera, small Dolomedes spiders and Large Dolomedes spiders, the Large Dolomedes spiders contained the highest levels of THg with an average concentration of 2.0±0.62 mg/kg. Small Dolomedes spiders had THg levels similar to that of OSM wetland Anisoptera with an average concentration of 1.4±0.33 mg/kg of THg (Figure 9). The two egg sacs belonging to the 2 Large Dolomedes spiders had lower Hg concentrations than the adults (1.6 mg/kg vs. 2.6 mg/kg for the adult and 1.2 mg/kg vs. 2.3 mg/kg for the adult). It has been reported previously that approximately 50% of the total mercury content in invertebrates is MeHg, while approximately 50% is

Hg^{2+}(Canadian Council of Ministries of the Environment, 2003). Studies show that in invertebrates containing concentrations of Hg^{2+}, effect concentrations (EC_{50}) in invertebrates range from 1.28 to 12 μgHg/L or 0.00128 mg/kg of Hg^{2+} to 0.012 mg/kg of Hg^{2+}, respectively (Canadian Council of Ministries of the Environment, 2003). Assuming that half of the THg in the studied invertebrates from the OSM wetland is Hg^{2+}, all invertebrates analyzed are well above that range, indicating that they are well above EC_{50} ranges for Hg^{2+}content.

THg concentrations and corresponding %LOI values in OSM wetland sediment ranged widely between quadrants. Q2 showed the highest levels of THg concentrations, with an average of 314.9±17.8mg/kg followed by Q4 with an average of 210.5±25.8 mg/kg of THg, Q1 with an average of 104.4±8.8 mg/kg of THg and, lastly, Q3 with and average of 22.7±1.4 mg/kg of THg (Table 1). All quadrants contained sediment mercury concentrations significantly higher than the Canadian Council of Ministers of the Environment Canadian Environmental (CCME) Quality Guidelines for freshwater sediment for the protection of aquatic life probable effect levels (PEL) values of 0.486 mg/kg of mercury in sediment (Canadian Council of Ministries of the Environment, 2003). Any level higher than the PEL value listed, typically means that in more than 50% of cases, adverse biological effects are likely occur (Canadian Council of Ministries of the Environment, 2003). %LOI showed a strong positive correlation (R^2 value of 0.97) with THg concentrations in Anisoptera (Figure 10). %LOI showed a less positive correlation with THg concentrations in Zygoptera (Figure 11) and Dolomedes (Figure 12) with R^2 values of 0.25 and 0.14, respectively.

Discussion

Even decades of Montague Gold Mining District being closed, our data reveals that mercury levels in sediment at the OSM wetland are well above the CCME freshwater sediment values. Mercury contamination within the sediment still has potential to bioaccumulate within organisms, especially in sediment dwelling invertebrates. Wetlands are stagnant areas of water, however flooding or other disturbances certainly have the potential to carry contaminants into neighbouring streams and other aquatic environments. With the exception of Q3, there seemed to be a trend indicating that higher levels of sediment THg corresponded with higher %LOI values. Higher %LOI showed a strong positive correlation between THg concentrations in Anisoptera and their corresponding quadrant. This suggests that organic matter plays a large role in mercury bioaccumulation in Anisoptera. Given that %LOI showed weaker positive correlations with THg concentrations in Zygoptera and Dolomedes when compared to their corresponding quadrants of collection, additional variables are likely at play that contribute to THg accumulation in these organisms.

The substantially higher THg concentrations in OSM wetland odonates compared with THg concentrations of RS odonates, provides strong evidence to suggest that odonates are bioaccumulating mercury within the wetland. Additionally, when compared to a study conducted on mercury bioaccumulation in four different dragonfly species collected from two lakes in Kejimkujik National Park where dragonfly nymphs showed a mean THg concentration of 0.251±0.117 mg/kg, OSM wetland dragonfly nymphs showed substantially higher THg (Figure 13) with a mean concentration of 1.38 ±0.240

mg/kg (Buckland-Nicks, 2013). This figure indicates that dragonfly nymphs contain almost 6 times the concentration of dragonfly nymphs found in the Kejimkujik Study. This comparison is especially significant due to the fact that Kejimkujik National Parks lakes have been found to have fish and loons with exceptionally high mercury levels (Page & Murphy, 2003). Due to the nature of the Odonate lifecycle and its potential to spread contamination not only to terrestrial landscapes, but also to other aquatic habitats, mercury contamination of Odonates is critical. Assuming THg is 50% Hg^{2+}(Canadian Council of Ministries of the Environment, 2003), Hg^{2+} found in OSM wetland Odonates exceed CCME criteria for Hg in aquatic biota, which further supports mercury contamination in these invertebrates. Upon emergence from mature nymphs to adults, adult Odonates fly away from the pond from which they emerged until sexual maturity has been reached which can be several days to weeks later (Corbet, 1980). This time spent away from the initial aquatic habitat from which they emerged makes emergent insects particularly vulnerable to terrestrial predators including bats, birds and amphibians (Corbet, 1980). Due to the re-location behaviour during the transitional stage from aquatic nymph to adult, and the fact that most Odonates don't return to the same aquatic waters from which they emerged, this only furthers the spread and geographical impacts of mercury contamination from one aquatic water body to the next. In addition to their relocation behavior that can contribute significantly to the spread of mercury to different aquatic habitats, there are many terrestrial organisms that feed exclusively on aquatic invertebrates, which can further contribute to the spread of mercury and its potential biomagnification. For example, certain species of bats have a diet consisting primarily of emerging aquatic insects. The large spatial distribution of such bats only

further supports mercury transfer to wider geographical areas (Little et al. 2015). Apart from aquatic insects acting as vectors for transmission of mercury from water to land, movement of contaminants between water bodies is also a great concern. For example, anadromous fish, such as the pacific salmon can also carry and spread contaminants from one water body to the next during migration having the potential to transfer mercury to terrestrial animals that eat fish as their primary diet, as well as to other aquatic invertebrates that feed on the carcasses of these fish (Mazeika et al. 2012)

One study conducted on the feeding behaviour of Dolomedes spiders suggests a large portion of their diet consists of Odonates (Zimmerman and Spence, 1989). Aquatic spiders have also been shown to contain some of the highest concentrations of mercury compared to other invertebrates (Sullivan & Rodewald, 2012). Given that mercury levels in large Dolomedes spiders were the higher than the Odonates studied, this supports the potential that biomagnification of mercury could also be occurring within the food chains at the OSM wetlands. This is of major significance given that spiders are food to many terrestrial organisms, thereby further increasing potential biomagnification.

Parental transfer of Hg to eggs or young has been extensively studied for fish with findings indicating that diets of maternal adults are the primary source of mercury transfer to fish eggs (Hammerschmidtt et al. 2005). However, this subject has been very rarely studied in insects. The discovery of two Dolomedes spiders with egg sacs in our sample set allowed us the opportunity to examine the differences in Hg concentrations between the adult and its eggs. The egg sacs had elevated Hg levels, which were slightly lower than those of their respective maternal spiders, indicating that there may be significant Hg transfer to young in spiders. A sample size of two means that we cannot

derive strong conclusions from this result, but it does indicate several important questions for future studies, including inter-generational transfer of Hg in addition to aquatic – terrestrial transfer of Hg from this contaminated site. Given the results, it is clear that the impacts go beyond the boundaries of the Old Stamp Mill wetland.

Conclusion

According to the data that half of the THg in the invertebrates is Hg^{2+}, provided by Canadian Council of Ministers of the Environment's Canadian Environmental Quality Guidelines for the protection of aquatic life, all mercury in invertebrates of focus are well above PEL values, and also well above EC_{50} ranges for Hg^{2+}content in invertebrates. This is of concern in regards to the quality of habitats within the OSM wetland.

When comparing THg concentrations of Odonates from the OSM wetland to concentrations of THg in Odonates at the RS and published data for Odonates from Keji, there is strong evidence to suggest that mercury is bioaccumulating in the organisms at the OSM wetland. Given that a large portion of Dolomedes spider diets include o\Odonates, and the OSM wetland Dolomedes have higher average THg levels than the OSM wetland Odonates, there is evidence to suggest the mercury could be bioaccumulating up through the food web. This is also significant given the potential for vector transmission to other aquatic habitats by prey organisms of these invertebrates, as well as to terrestrial habitats by terrestrial organisms that rely on aquatic invertebrates as a diet source.

The wetland sediment is highly contaminated with concentrations of THg well above Canadian Environmental Quality guidelines for freshwater sediment, indicating that mercury from tailing material has the potential to pose a significant affect on aquatic wildlife living within contaminated waters/sediments.

While there seems to be a strong correlation between organic matter in sediment and THg levels in Anisoptera, correlations were weaker between THg concentrations in

Zygoptera and Dolomedes and organic matter contained in the sediment. It is likely that other variables play a role in THg invertebrate accumulation. These variables should be further considered and investigated.

References:

Bates, J., L., E. (1987) *Gold in Nova Scotia.* Halifax, Nova Scotia: Nova Scotia Department of Government Services, Information Services Division, Publishing Section.

Buckland-Nicks, A., Hillier, K.N., Avery, T.S., O'Driscoll, N.J. (2014) Mercury Bioaccumulation in Dragonflies (Odonata: Anisoptera): Examination of Life Stages and Body Regions. *Environmental Toxicology and Chemistry.* 33(9): 2047-2054

Burns, J.C., & Reitzammer, A. (2009) Dragonfly Invasion. *Science Activities.* 27(4): 8

Canadian Council of Ministers of the Environment. 2001. Canadian sediment quality guidelines for the protection of aquatic life: Introduction. Updated. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

Canadian Council of Ministers of the Environment. 2003. Canadian water quality guidelines for the protection of aquatic life: Inorganic mercury and MeHg. In: Canadian environmental quality guidelines, 1999. Canadian Council of Ministers of the Environment, Winnipeg.

Chapman, E.V., Robinson, J., Berry, J., Campbell, L.M. (2015) Can low-dose selenium (Se) additive reduce environmental risks of mercury (Hg) and arsenic (As) in old goldmine tailings? *Saint Mary's University, Department of Environmental Science and Amec Foster Wheeler Environment and Infrastructure.* Personal Data.

Clifford, H.F. (1991) Aquatic Invertebrates of Alberta. Edmonton, AB, Canada. The University of the Alberta Press.

Colombo, M., Ha, J., Reinfelder, J., Barkay, T., Yee, N. (2013) Oxidation of Hg(0) to Hg(II) by diverse anaerobic bacteria. *Chemical Geology.* 356: 334-340

Corbet, P.S. (1980) Biology of Odonata. *Ann. Rev. Entomol.* 25: 189 – 217.

Dong, W., Liang, L., Brooks, S., Southworth, G., Gu, B. (2009) Roles of Dissolved Organic Matter in the Speciation of Mercury. *Environmental Chemistry.*

Gilmore, C.C., Henry, E.A., Mitchell, R. (1992) Sulfate stimulation of mercury methylation in freshwater sediments. *Environmental Science and Technology.* 26(11): 2281-2287

Gochfeld, M. (2003) Cases of mercury exposure, bioavailability and absorption. *Ecotoxicology and Evironmental Safety* 56(1): 174-179

Hammerschmidtt, C.R. & Sandheinrich, M.B. (2005) Maternal diet during oogenesis is the major source of MeHg in fish embryos. *Environmental Science and Technology.* 39(10): 3580-3584

Heiri, O., Lotter, A.F., & Lemcke, G. (2001) Loss on ignition as a method for estimation organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of paleolimnology.* 25: 101 – 110

Hill, J.R., O'Driscoll, N.J., Lean, R.S. (2009) Size Distribution of MeHg associated with particulate and dissolved organic matter in freshwaters. *Science of the Total Environment.* 408(2): 408-414

Hopkins, C.S., & Vallino, J.J. (2005) Efficient export of carbon to the deep ocean through dissolved organic matter. *Nature.* 433: 142 – 145.

Hsu-Kim, H., Kucharzyk, K., Zhang, T., Deshusses, M. (2013) Mechanisms Regulating Mercury Bioavailability for Methylating Microorganisms in the Aquatic Environment: A Critical Review. *Environmental Science and Technology.* 47(6): 2441-2456

Jiang, Y., Cizdziel, J., Lu, D. (2013) Temporal patterns of atmospheric mercury species in northern Mississippi during 2001-2012: Influence of sudden population swings. *Chemosphere.* 93(9): 1694-1700

Little, M.E., Burgess, N.M., Broders, H. G., and Campbell, L.M. (2015) Mercury in Little Brown Bats (**Myotis lucifugus**) Maternity Colonies and Its Correlation with Freshwater Acidity in Nova Scotia, Canada. *Environmental Science and Technology.* 49: 2059-2065.

Mazeika, S., Sullivan, P., Rodewald, A.D. (2012) The Energetic Pathways That Move Contaminants From Aquatic to Terrestrial Environments. *Environmental Toxicology and Chemistry.* 31(6): 1175-1183

Mazeika, S., Sullivan, P., and Rodewald, A.D. (2012) In a state of flux: The energetic pathways that move contaminants from aquatic to terrestrial environments. *Envrionmental Toxicology and Chemistry.* 31(6): 1175 – 1183.

Merritt, R.W. & Cummins K.W. (1984) An Introduction to the Aquatic Insects of North America. U.S.A. Kendall/Hunt Publishing Company.

Page, K.D., & Murphy, B.J. (2003) The geological sources of Hg contamination in Kejimkujik National Park, Nova Scotia, Canada: A GIS approach. *Environmental Geology.* 43: 882 – 891

Parsons, M.B.., & Little, M.E. (2015) Establishing geochemical baselines in forest soils for environmental risk assessment at the Montague and Goldenville gold districts, Nova Scoita. *Atlantic Geology.* 51: 362-386

Pirrone, N., & Mahaffey, K.R. (2005) Dynamics of Mercury Pollution on Regional and Global Scales. © *Springer Science +Business Media Inc. New York, New York.* Chapter 1, pp. 1-2.

Province of Nova Scotia. (2011). *Forest Vegetation Types – FP6.* http://novascotia.ca/natr/forestry/veg-types/fp/fp6.asp

Pritchard, G. (1964) The Prey of Dragonfly Larvae (Odonata; Anisoptera) in Ponds in Northern Alberta. *Canadian Journal of Zoology.* 42(5): 785 – 800

Ravichandran, M. (2004) Interactions between mercury and dissolved organic matter – a review. *Chemospehere.* 55(3): 319-331

Saunders, J.R., Knopper, L. D., Koch, I., Reimer, K.J. (2010) Arsenic transformations and biomarkers in meadow voles (*Microtus pennsylvanicus*) living on abandoned gold mine sit in Montague, Nova Scotia, Canada. *Science of The Total Environment.* 408(4): 829- 835

Selvendeiran, P., Driscoll, C.T., Bushey, J.T., Montesdeoca, M.R. (2007) Wetland influence on mercury fate and transport in a temperate watershed. *Environmental Pollution.* 154(1): 46 – 55

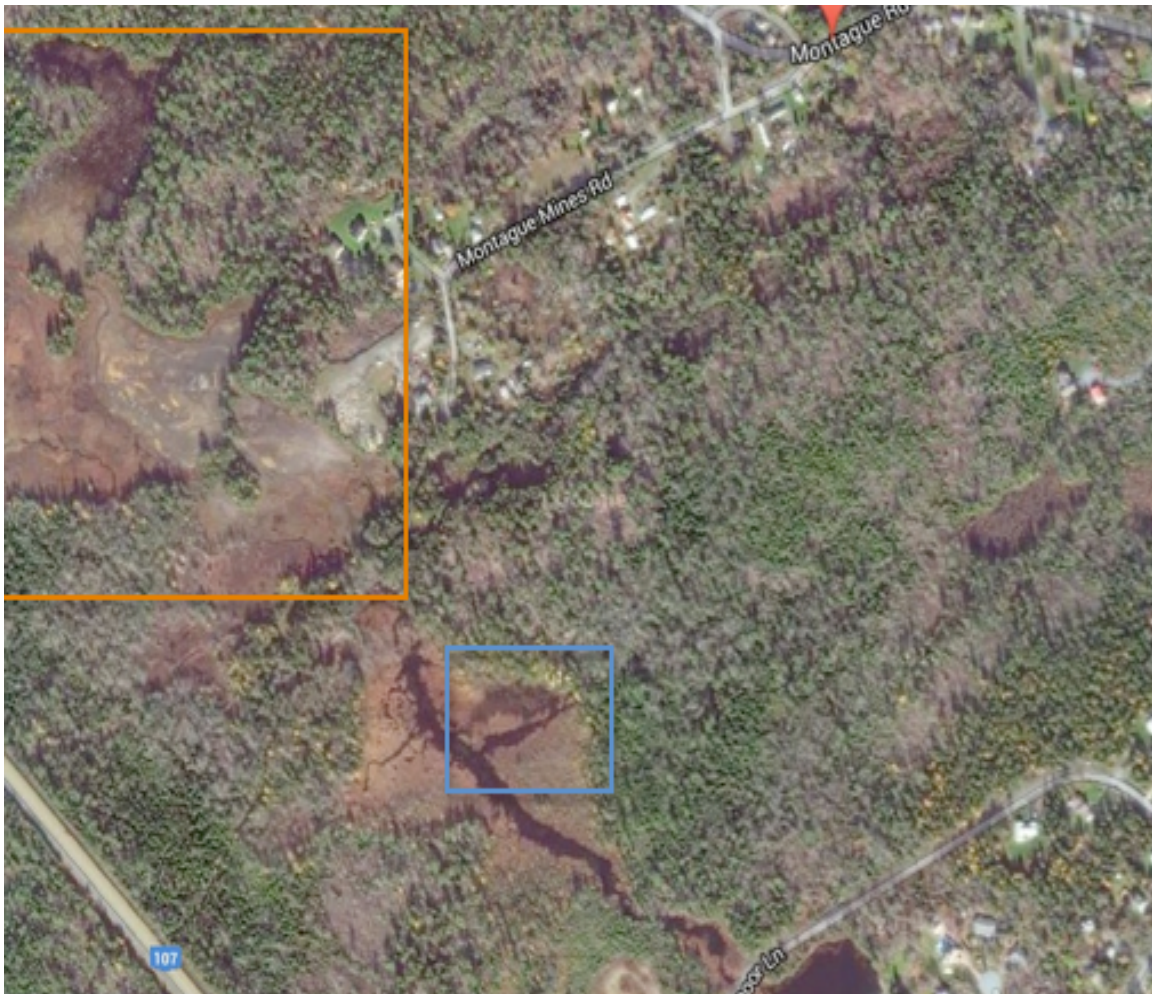
U.S EFA. (2001) Methods of collection, storage and manipulation of sediments for Chemical and Toxicological Analyses: Technical Manual. EPA 823-B-01-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Weber, L. (2003) Spiders of the North Woods. Duluth, MN, U.S.A. Kollath-Stensaas Publishing.

Wright, A.L, & Reddy, K.R., (2009) Dissolved Organic Matter in Wetlands. *Soil and Water Science Department, UF/IFAS Extension.* SL 294

Zimmerman, M. & Spence, J.R., (1989) Prey use of fishing spider Dolomedes triton (Pisauridae, Araneae): An important predator of the neuston community. *Oecologia.* 80: 187 – 194

Tables and Figures



(Captured from Google Images in June 2015)
Figure 1: Areal view of Montague Gold Mining District. The orange boarder indicates the main source of tailing material. The blue border indicates the wetland.

(Source:https://museumofindustry.novascotia.ca/nova-scotia-industry/gold-mining/impact-environment)

Figure 2: The main tailing-dumping site, approximately 700 meters from the wetlands. Areas of grey soil indicate exposed tailing material.

Photo Credit: Linda Campbell

Figure 3: Photo of the Old Stamp Mill wetland taken from the shoreline along Quadrant 1 (see Figure 4). Vegetation is largely spaced out and brownish-green in colour, with an increase in green vegetation closer to the Brook.

(Captured from Google Images in June 2015)
Figure 4: The division of the Old Stamp Mill wetland into 4 quadrants. Each quadrant was roughly 15 meters by 15 meters in area. Q1 is closest to the tailing waste outflow, while Q3 is closest to Mitchell Brook, seen in the bottom left-hand corner.

Figure 5: Collection of invertebrates in the Old Stamp Mill wetland.

Figure 6: Labeled vials containing wetland invertebrates being grouped according to physical similarities and characteristics.

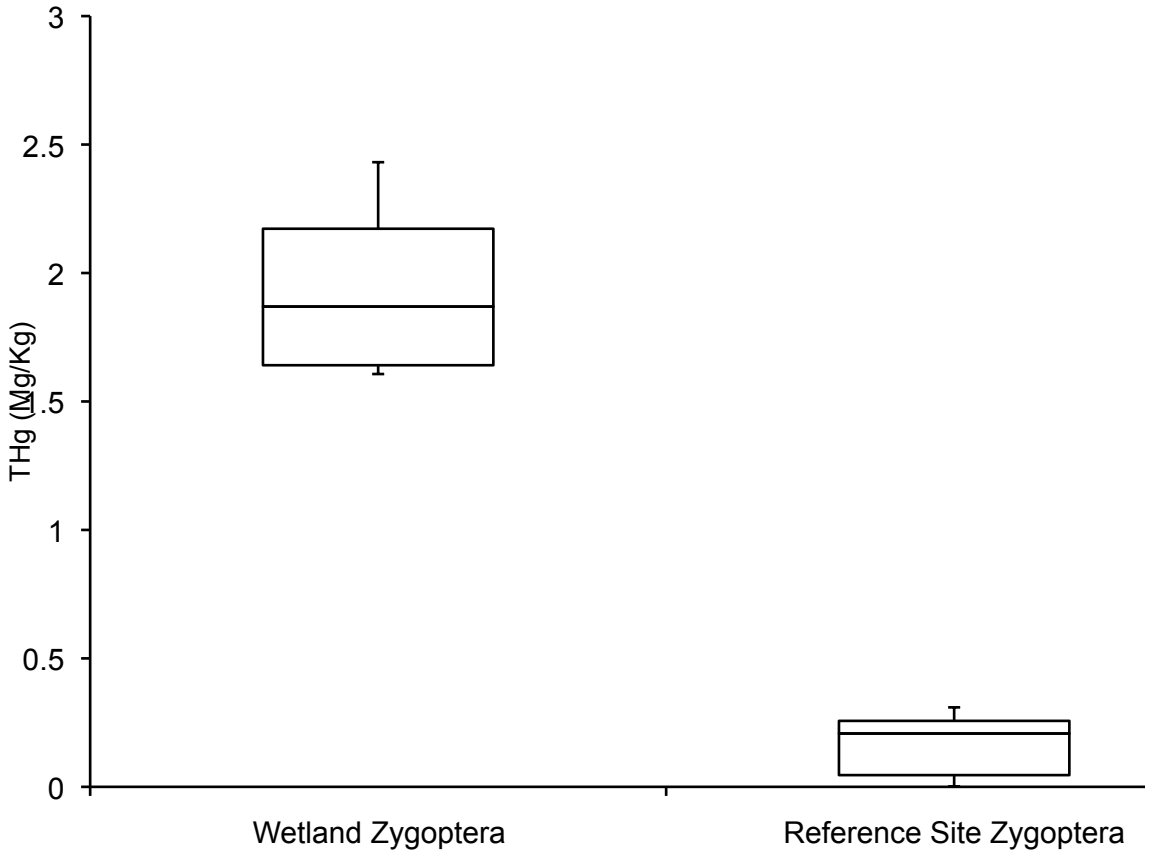


Figure 7: Average THg concentrations for Old Stamp Mill (OSM) Zygoptera (1.9±0.4 mg/kg, n=52), and Reference Site Zygoptera (0.17±0.13 mg/kg, n=31).

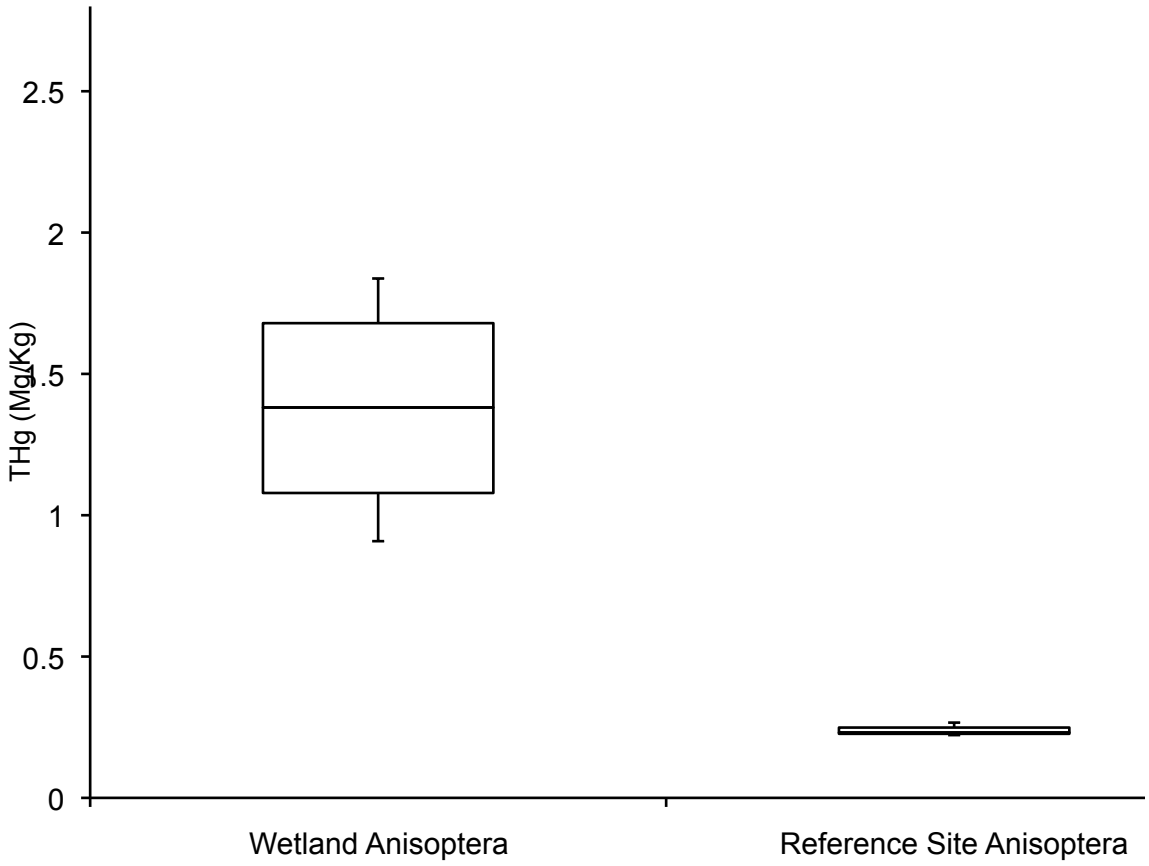


Figure 8: Average THg concentrations for Old Stamp Mill (OSM) Anisoptera (1.4±0.24 mg/kg, n=19) and Reference Site Anisoptera (0.24±0.023 mg/kg, n=16)

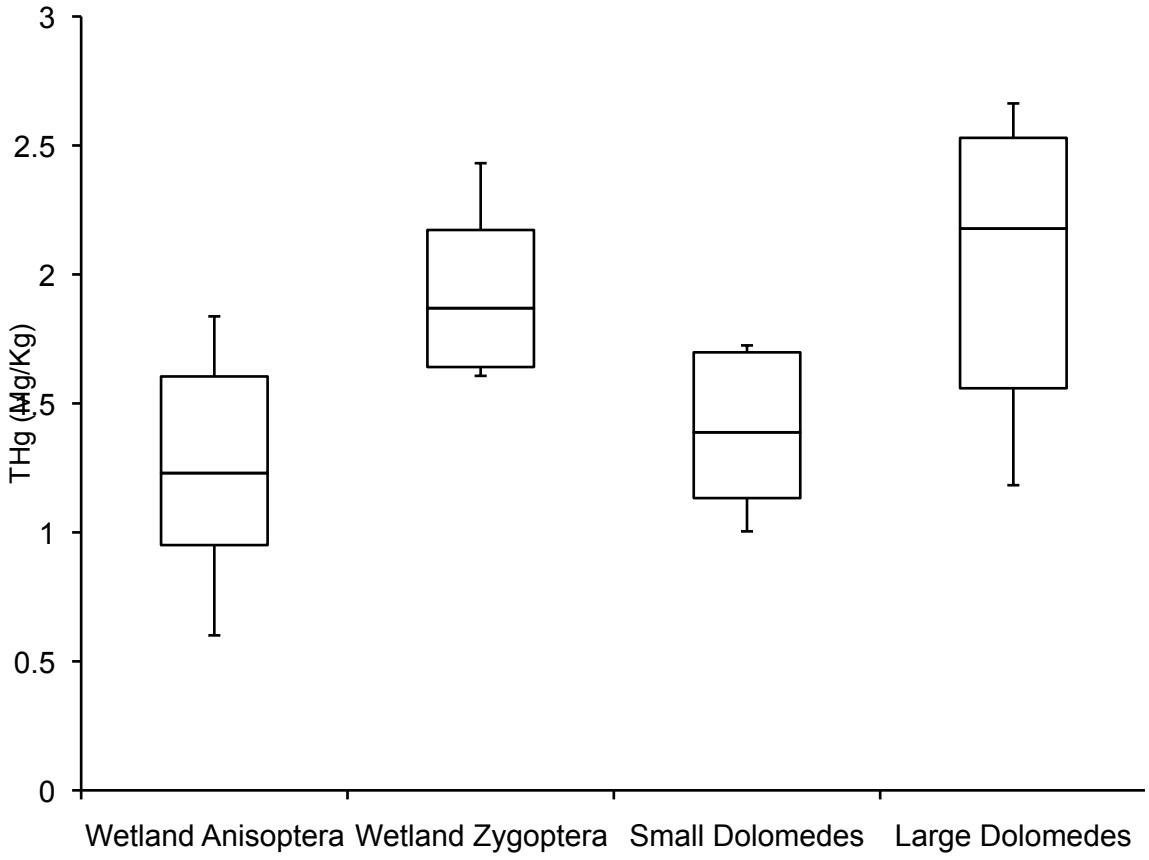


Figure 9: Average THg concentrations for Old Stamp Mill (OSM) Anisoptera (1.4±0.24 mg/kg, n=19), average THg concentrations for Old Stamp Mill (OSM) Zygoptera (1.9±0.4 mg/kg, n=52), average THg concentrations for Old Stamp Mill (OSM) Large Dolomedes spiders (2.0±0.62 mg/kg) and average THg concentrations for Old Stamp Mill (OSM) small Dolomedes spiders (1.389±0.325 mg/kg)

Quadrant	THg (Mg/Kg)	%LOI
1	104.42 (± 8.80)	3.89 (± 0.035)
2	314.88 (± 17.76)	20.78 (± 1.17)
3	22.71 (± 1.39)	77.63 (± 1.44)
4	210.52 (±25.75)	14.54 (± 0.748)

Table 1: Average THg and %LOI values for3 sub-samples of sediment collected from the centroid of each Old Stamp Mill wetland quadrants.

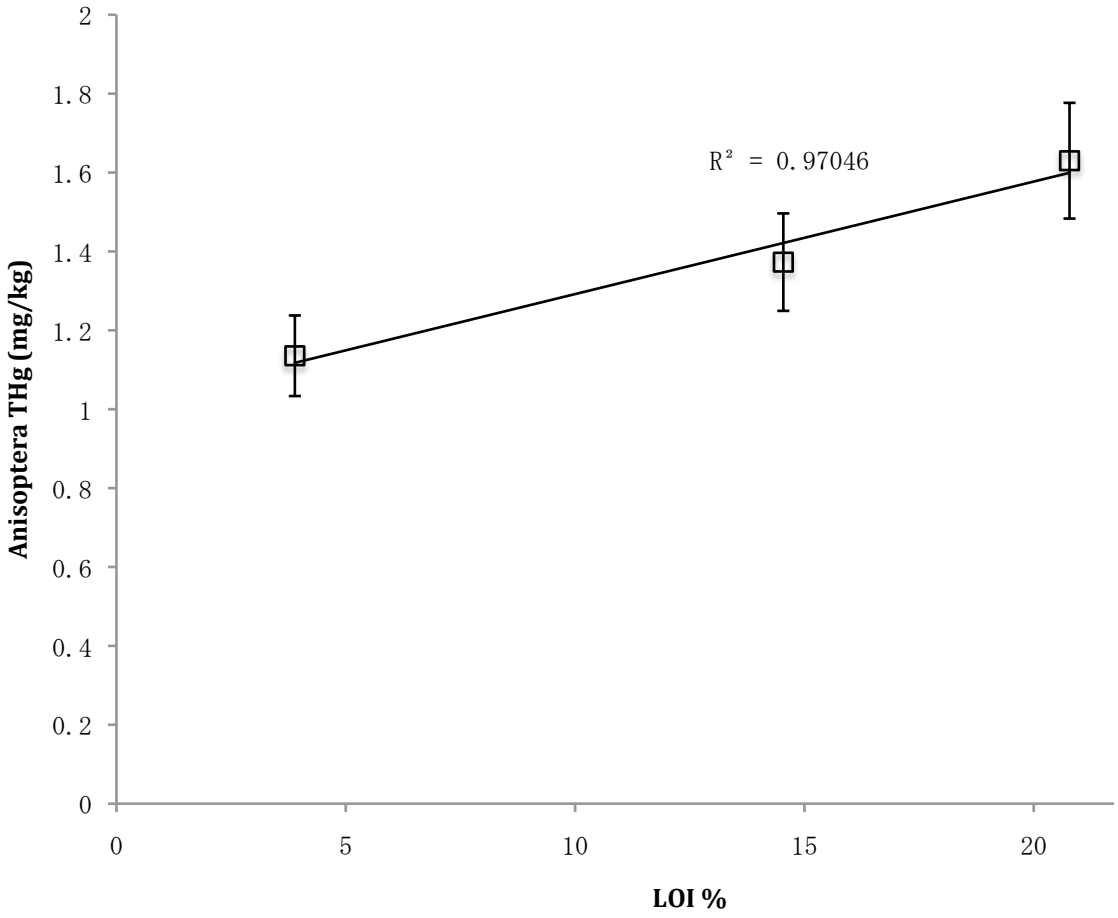


Figure 10: Anisoptera THg concentrations vs. their corresponding quadrant %LOI of sediment analysis. No Anisoptera data available for Q3.

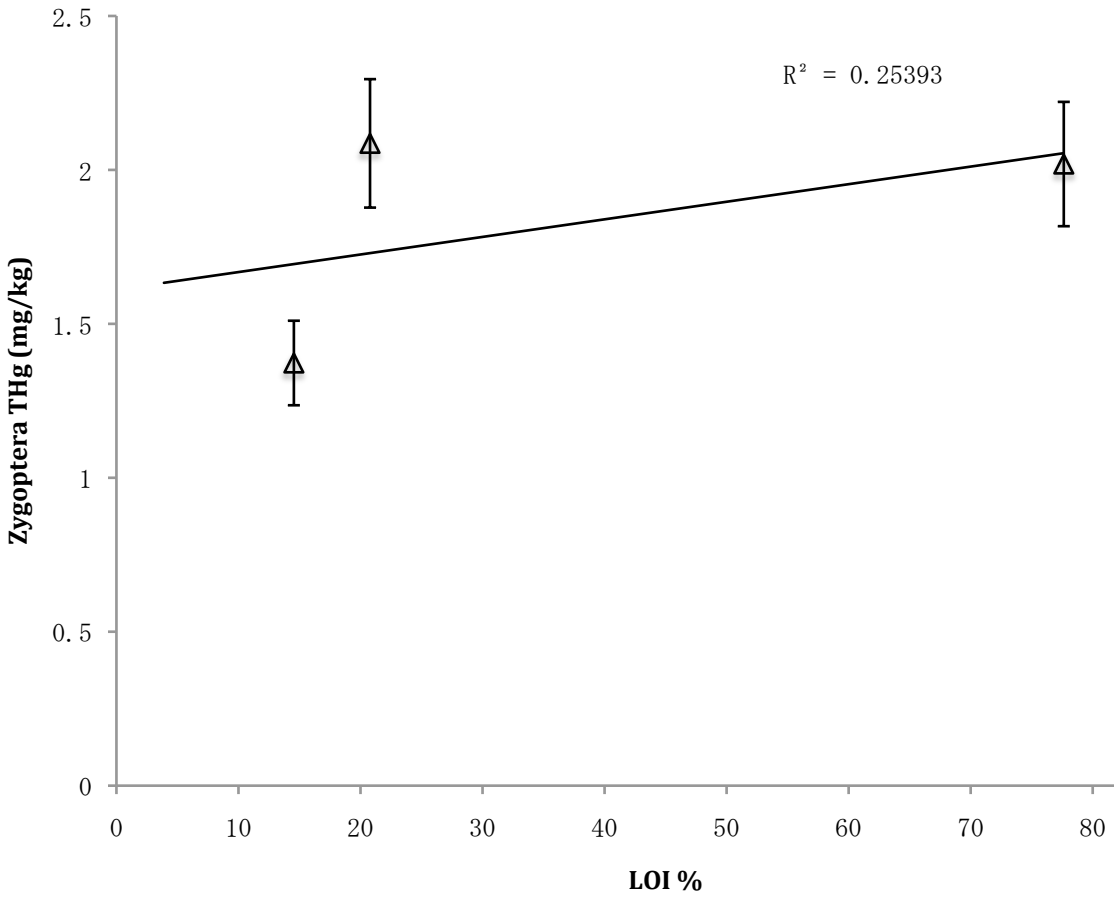


Figure 11: Zygoptera THg concentrations vs. their corresponding quadrant %LOI of sediment analysis. No Zygoptera data for Q1.

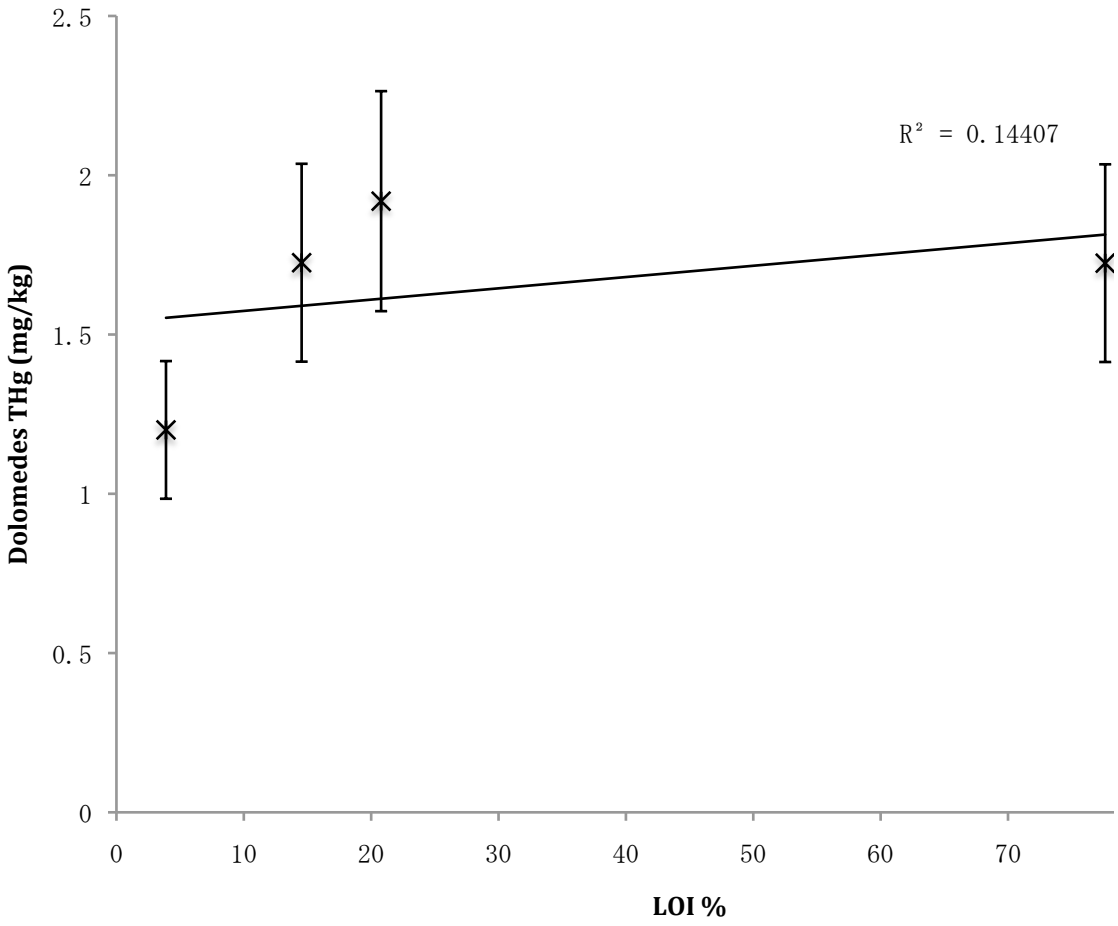


Figure 12: Dolomedes THg concentrations vs. their corresponding quadrant %LOI of sediment analysis.

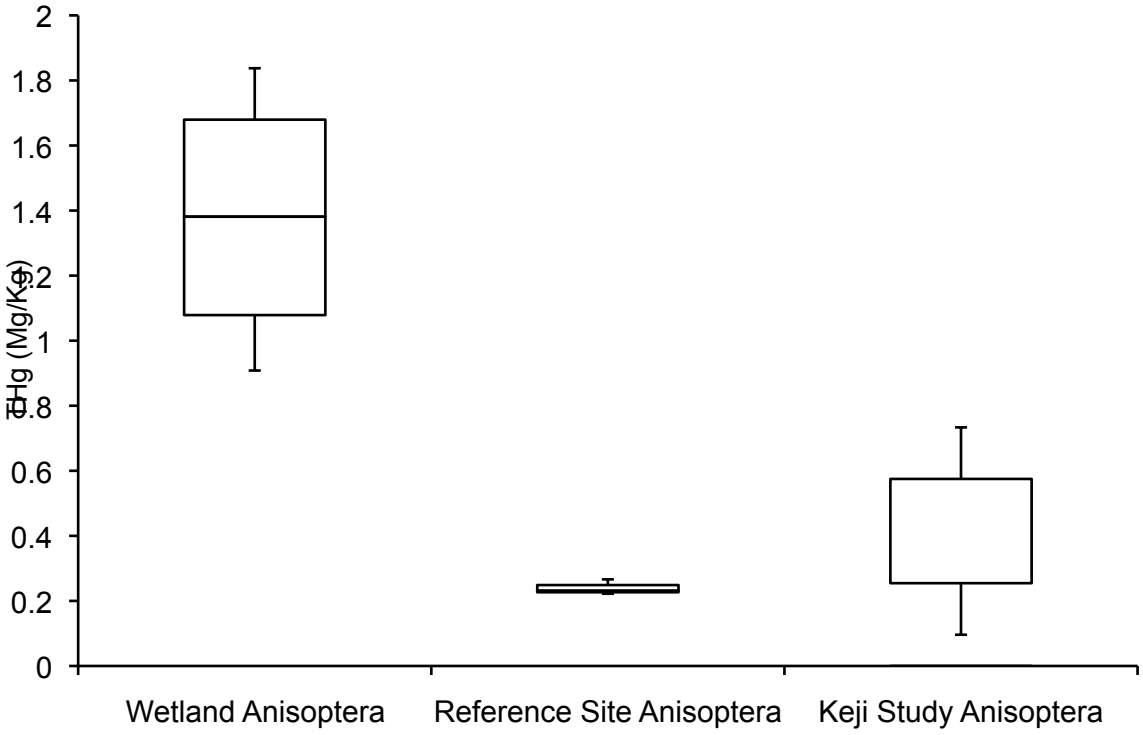


Figure 13: Average THg concentrations for Old Stamp Mill (OSM) Anisoptera (1.4±0.24 mg/kg, n=19), average THg concentrations for Old Stamp Mill (OSM) Zygoptera (1.9±0.4 mg/kg, n=52), Average THg concentration for Keji Study (0.251±0.117 mg/kg; (Buckland-Nicks, 2013).