Bioaccumulation of Contaminants in Amphibians in Historical Gold Mining Areas of Nova Scotia

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Abstract

Nova Scotia has a long history of gold mining dating back to the mid-1800's. Early mining techniques depended on the use of liquid mercury (Hg). As there were little or no environmental regulations at the time, over 3,000,000 tonnes of mine waste (tailings) were released into the environment. These tailings can contain high concentrations of toxic chemicals including Hg and arsenic (As). Such chemicals have negative effects on the environment and on wildlife. Frogs are often used as indicators of environmental conditions. Their thin, permeable skin and semi-aquatic lifestyle can lead to the bioaccumulation of toxins in frog tissues. This study compares bioaccumulation of Hg in frogs collected from six research sites and one reference site from within historical gold mining districts in Nova Scotia, Canada. Dried, ground frog-leg tissues were analyzed for Hg content using a Milestone DMA 80 direct mercury analyzer. This project tests the hypothesis that if concentrations of Hg are high at research sites identified as legacy tailing areas in Nova Scotia, then the concentrations of total mercury content (THg) in the tissues of amphibians collected at the respective sites will also be high. Adult amphibians sampled from contaminated sites had concentrations of THg ranging from 0.1324 - 2.1329 mg/kg. Tadpoles sampled from contaminated sites had concentrations of THg ranging from 0.1384 -15.9412 mg/kg. The THg concentrations in adult amphibians at a reference site ranged from 1.664 – 2.3959 mg/kg. The THg concentrations in tadpoles at the reference site ranged from 0.0494 – 3.3312 mg/kg. These ranges indicate elevated Hg levels in both adult amphibians and tadpoles. This project, as part of the legacy gold mine contaminants research led by the Dynamic Environment and Ecosystems Health Research (DEEHR) team at Saint Mary's University will enhance the understanding of impacts of historical human activity on aquatic ecosystems.

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1.0 Introduction

1.1 Gold Mining in Nova Scotia

Nova Scotia has a history of gold mining dating back to the mid-1800's. Although there were small, unofficial gold sightings prior to this, the first official discovery of gold in Nova Scotia was in 1858 in Mooseland on the Tangier River. While moose hunting, Captain L'Estrange, a British army officer, discovered gold in quartz rocks; however, at the time he did little more than discover it (Bates, 1978). It was a few years later (1861) that a farmer named John Gerrisb Pulsiver from Musquodoboit searched around the same area as Captain L'Estrange and found gold in quartz veins and boulders. It was this discovery that kickstarted Nova Scotia's first gold rush. Many individuals rushed to the Eastern shore to try to stake out their claim in Nova Scotia's gold. More discoveries were found in and around Lawrencetown, Ovens, Goldenville, and Waverly (Bates, 1978). Increasing interest in gold mining at the time resulted in the government stepping in and establishing new mining districts. Over the subsequent years, 64 mining districts (Figure 1) were established across Nova Scotia, which together would contain hundreds of individual mines (Bates, 1978). There have been three gold rush periods in Nova Scotia's history, the first from 1861- 1876, the second from 1882-1903, and the third from 1935 - 1943 (Nova Scotia Archives, 2013).

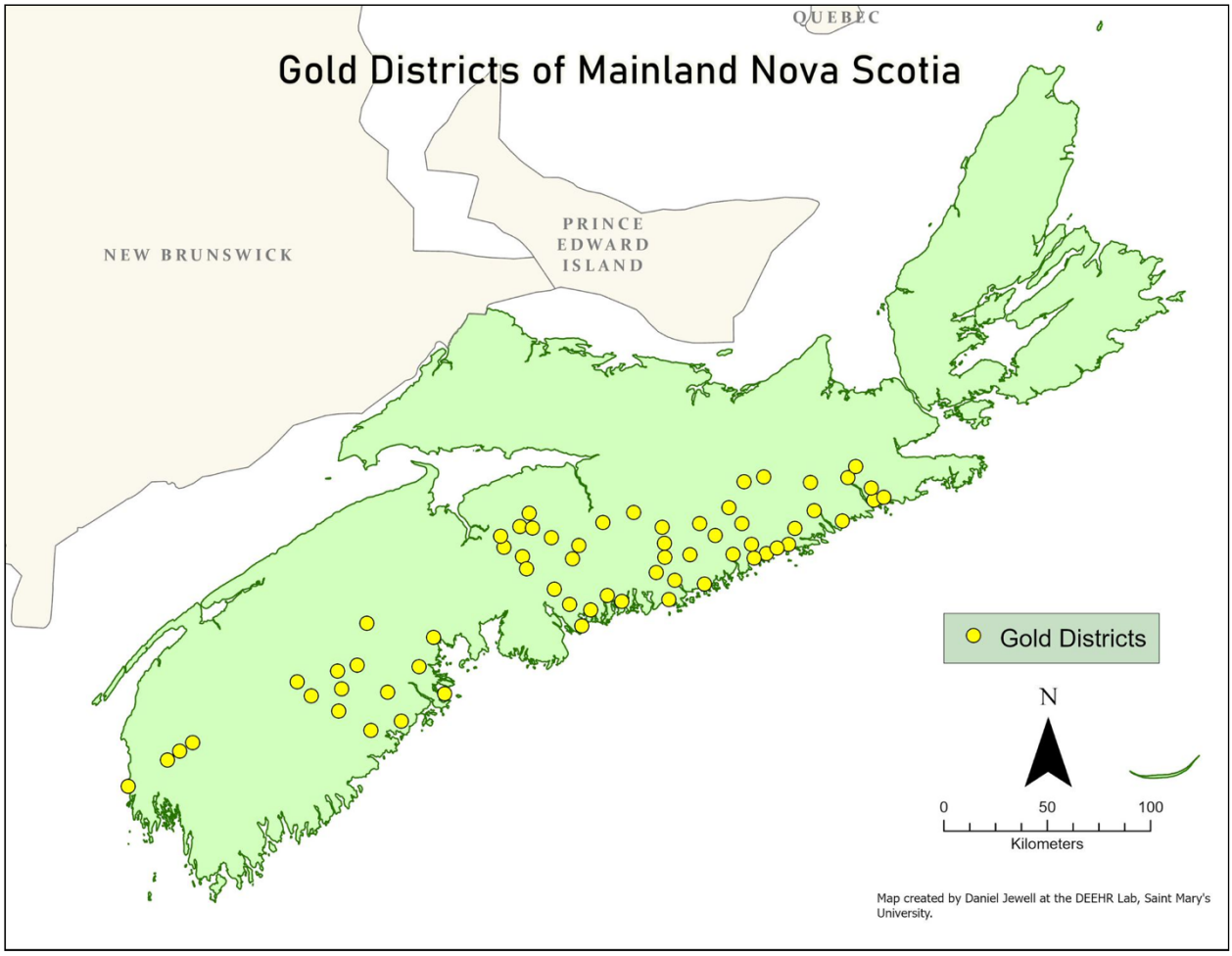

Figure 1. Map of gold districts of mainland Nova Scotia. Jewell (2021).

During the first gold rush, miners used simple tools such as pickaxes and shovels (Bates, 1978). Gold was extracted using stamp mills and mercury (Hg) amalgamation techniques. In this process, quartz rocks containing gold were excavated and brought into shaft houses. The rocks were transported downhill to stamp mills where large stamps weighing up to 1,000 lbs were used to press the rock into a fine sand-like material. Freshwater was then pumped in to wash the sand over copper plates coated with Hg. Small pieces of free gold would stick to the Hg creating an amalgam. The amalgam was scraped from the copper plates and the Hg was boiled off leaving

small amounts of gold (Bates, 1987). It is estimated that for every ounce of gold recovered one ounce of Hg was used. In this process it is estimated that 10-25% of the Hg used was lost to the environment (Parsons, et al., 2012). Despite use of primitive techniques, lack of knowledge and general inefficiencies in the early days, the first gold rush produced approximately 208,942.85 troy ounces (t oz) of gold (Art Gallery of Nova Scotia, 2013). It has also been documented that these estimates may be low as production volumes from gold mining were often under-reported to avoid governmental fees (Art Gallery of Nova Scotia, 2013).

Techniques to extract gold became more specialized and methodical during Nova Scotia's second gold rush. More gold mining companies sprung up and fewer independent miners were looking for gold (Bates, 1987). In this period, miners started using dynamite, which allowed them to dig deeper and ultimately increase production. In the 1880's a gold extraction process called cyanidation was introduced and used in association with the Hg amalgamation process (Bates, 1987). This process uses cyanide to dissolve gold within the rock and draws the gold out in a liquid form that is treated, or leached, to remove cyanide. From inception, cyanidation has been controversial due to the poisonous nature of cyanide and the threat it poses to the environment. During the second gold rush, approximately 497,842.75 t oz of gold was produced (Art Gallery of Nova Scotia, 2013).

Nova Scotia's third gold rush is attributed to an increased demand for arsenic (As) in the 1920's prompting a search for arsenopyrite, a mineral associated with gold deposits, which in turn sparked a renewed interest in the search for gold (Bates, 1987). Other significant world events factored into the third gold rush including the stock market crash in October 1929 and the

Great Depression of the 1930's (Art Gallery of Nova Scotia, 2013). During the third gold rush approximately 20,000 ounces of gold was extracted each year and, in total, approximately 154,319.7 t oz of gold was produced (Art Gallery of Nova Scotia, 2013).

Between 1862 and the mid-1940's, it is estimated that over 1.2 million t oz of gold was produced from Nova Scotia's historical gold mining sites (Parsons et al., 2012). Mine waste, also known as "tailings", from many decades of gold mining in Nova Scotia contain high concentrations of Hg, As, and other materials that occur naturally in the ore (Parsons et al., 2012). Most of the gold found in Nova Scotia was extracted from quartz rocks (Art Gallery of Nova Scotia) which naturally contain sulfide arsenopyrite (FeAsS). Crushing quartz to extract gold oxidizes the FeAsS releasing the As found in tailings. Over 3,000,000 tonnes of tailings were generated throughout these three gold rush periods (Parsons et al., 2012). There were no environmental regulations at the time, and therefore most of the tailings were dumped without care into rivers, lakes, and wetland areas surrounding the mill sites (Parsons et al, 2012). As a result, tailings flats—which can span as large as 1 km^2 in surface area and go down as deep as a few meters—can be found at almost all mining sites. Over time, the tailings have migrated downstream via rivers and streams and have been found up to 2 km away from their original site (Drage, 2015). Although most of the historical gold mining districts have been long abandoned, Hg and As do not degrade and the tailings produced from mining over a period of 100+ years have a significant impact on the environment today (Drage, 2015).

Gold mining is still active in some parts of Nova Scotia, although the amount of gold produced does not compare to any of the past gold rushes. In the early 2000's increasing gold

prices sparked interest in exploration and new gold mines started to appear (Nova Scotia Archives, 2013). Gold mining, however, has become a highly regulated process in Nova Scotia whereby prospective mining companies must invest in a years-long process which includes scientific studies to control current and future environmental impacts (Art Gallery of Nova Scotia, 2013). Unfortunately, today's regulations cannot eliminate the legacy of historical gold mining littered across Nova Scotia. Many mine shafts remain open and abandoned and, of course, the environmental impact of tailings will be a reality for years to come.

1.2 Environmental Impacts of Gold Mining

Many forms of Hg are toxic, and methylmercury (MeHg), a common organic form of Hg is of particular concern. It can biomagnify and accumulate in organisms through food webs, particularly in wetland environments. Methylmercury is a neurotoxic chemical which has been found to impact embryo development and vertebrate nervous systems (Clark et al., 2021). Arsenic can form many different compounds (species), and its toxicity can vary depending on its speciation from non-toxic to extremely toxic (Reid et al., 2020). Excessive exposure to As in humans has been linked to multiple forms of cancer including cancer of the skin, liver, and lungs (Doe et al., 2017). With the recent development of land close to old mining sites, there is an increased interest in assessing the tailings and their potential risks to humans and the environment. The first environmental investigation on the subject was in 1976, in Waverly, where a resident suffered chronic As intoxication from well water (Drage, 2015). The well water was found to have As concentrations that were over 500 times higher than the Canadian drinking water guidelines.

Over the years, numerous studies have also been undertaken in Nova Scotia to assess the effects of gold mining. Extensive work has been done mapping out tailing areas, looking at the chemistry of the tailings as well as analyzing water, soil, and air samples in the surrounding areas. Parsons et al., (2012) provides a comprehensive report on sediment and surface water concentrations of 14 historical gold mining districts in Nova Scotia. Tailing flats have also been mapped in these areas, although these maps may represent only a small section of the total tailings area because these tests were only performed on Crown lands and not on private properties (Drage, 2015). Parsons, et.al., (2012) found that As concentrations at the 14 districts (n=482) ranged from 10 mg/kg to 312,000 mg/kg. Mercury concentrations ranged from 5 μg/kg to 350,000 μg/kg. The Canadian Environmental Protection Act, 1999 provides sediment quality guidelines to protect aquatic life and soil quality guidelines to protect the environment and human health. Over 99% of samples tested for As concentration exceeded both sediment and soil quality guidelines. Mecury concentration exceeded sediment quality guidelines in over 71% of the samples and soil quality guidelines in over 20% of samples (Parsons et al., 2012). Additionally, levels of dissolved As were found to be very high when impacted by gold mine tailings. These values ranged from 0.2 μg/L to 6580 μg/L, where values at reference sites were less than 25 μg/L. Dissolved Hg concentrations were found to be relatively low, ranging from 1.8 ng/L to 61 ng/L. These concentrations were found close to areas with tailings that contain high concentrations of Hg which suggests that most Hg is in insoluble forms (Parsons et al., 2012).

Environmental impacts of Hg due to historical mining practises is not unique to Nova Scotia. In the 19th century, Hg amalgamation techniques were used in California across the Sierra Nevada gold belt for hydraulic mining (Hunerlach et al., 1999). Hydraulic mining technology

involved the use of powerful canons to spray large quantities of water into the ground to wash away gravel deposits and break down the ore into a "slurry". The slurry was then sifted in troughs and Hg amalgamation was used in the troughs to recover gold (Hunerlach et al., 1999). Elevated concentrations of Hg have been found in the soil, water and the tissues of animals in these Californian mining areas. A sampling of the water and sediment was done in the late 1990s providing information on Hg levels still in these areas. Hunerlach et al., (1999), looked at the Hg contamination in the Dutch Flat mining district and found elevated concentrations of Hg in water and sediment samples. Unfiltered water samples from the area ranged from 45 to 10,400 ng/L Hg and sediment samples collected from a sluice box in the Dutch flat mining district had Hg concentrations ranging from 1,800 to 15,000 ng/g wet weight (ww) (Hunerlach et al., 1999).

Current mining practises, such as artisanal and small-scale mining (ASM), still depend on Hg. The term ASM encompasses miners from around the world who use basic tools to extract gold-bearing ore from the ground (Veiga et al., 2006). Artisanal and small-scale mining is the largest source of Hg pollution on Earth (Esdalie and Chalker, 2018). The ASM practice has grown over the past number of years, and it is estimated that in 2017, 40.5 million people were directly engaged in the industry of ASM (Fritz et al., 2018). Many ASM miners cannot independently process and extract gold from the ore. These miners rely on processing centres where the gold is extracted for a fee (Cordy et al., 2011).

In over 50 countries, the main ASM gold extraction technique is Hg amalgamation as it is viewed as a cheap and easy option. This view, however, may be short sighted. The Hg technique can be very inefficient as on average only 30% of the gold is recovered (Veiga et al.,

2009) and Hg contamination is a significant issue as 23-30% (approximately 1000 tonnes) of Hg is lost to the environment annually (Veiga et al., 2006, Esdaile and Chalker, 2018). The use of sulphate, chloride, or bromine are possible alternatives to Hg amalgamation (Veiga et al., 2009) but the Hg amalgamation process is so ingrained that miners are hesitant to make changes to their practices aimed at reducing Hg contamination, even when alternatives are more efficient for producing gold (Veiga et al., 2009).

1.3 Bioaccumulation

Metals and toxins, like Hg and As can enter and build up in the tissues of organisms. This process is called bioaccumulation. Bioaccumulation can occur in amphibians and fish through the skin or gills from direct intake of water, sediment, and soil from the environment (Franke et al., 1994). This can also happen through the ingestion of the chemicals, contaminated sediment, and food (Wang, 2016). Bioaccumulation does not always result in direct adverse effects on animals, such as mortality, but it can still pose a risk to health if ingested (Franke et al., 1994). Many studies have been done looking at the bioaccumulation of toxins in many organisms found in all parts of the world (Franke et al., 1994). Aquatic and semi aquatic animals, such as fish and amphibians, are often used in studies of bioaccumulation of metals. Such organisms can provide an indication of the contamination levels in the water as well as in the tissue of animals. In a study by Hunerlach et al., (1999) which looked at the water and sediment Hg levels in the hydraulic gold mining areas in California, fish were analyzed for Hg content. Elevated concentrations of Hg were found in the fish tissues ranging from 0.1 to 0.2 ppm (Hunerlach et al., 1999).

In Nova Scotia, many studies looking at the bioaccumulation of toxic metals in animals and plants have been carried out. For example, LeBlanc et al., (2019) looked at the bioaccumulation of Hg and As in aquatic invertebrates (dragonfly larvae, amphipod crustaceans, and aquatic feeing spiders) from five contaminated wetlands and two reference sites associated with legacy gold mine tailings. In all tailing wetlands the aquatic insect larvae and adult insects had elevated Hg concentrations when compared to the reference sites. The concentrations of Hg in dragonflies at the larval stage (n = 280) from the tailing sites ranged from 0.07 to 12.00 ppm, while they ranged from 0.001 to 1.15 ppm at the reference sites. At the adult stage in dragonflies (n= 180), concentrations of Hg ranged from 0.0001 to 6.38 ppm at the tailing sites, while at the reference sites the concentrations ranged from 0.00004 to 3.41 ppm (LeBlanc et al., 2019). When testing for As, larvae from the order Odonata had significantly higher concentrations of As at two of the impacted wetlands when compared to the reference sites. Arsenic concentrations ranged from 17 to 890 ppm, while at the reference sites they ranged from 8.8 to 20.0 ppm (LeBlanc et al., 2019).

Koch et al., (2007) investigated As speciation in *Mya arenaria* (soft shell) clams and seaweed collected from Seal Harbour, Nova Scotia. The concentration of As in the marine clams ranged from 218 to 228 ppm ww where the total As found in the reference clams ranged from 7.0 to 7.9 ppm ww (Koch et al., 2007). Total As in seaweed ranged from 6.0 – 10 ppm ww. The low concentration of As in the seaweed could be due to seaweed not taking up sediment in the same manner as do clams (Koch et al., 2007).

1.4 Bioaccumulation in Amphibians

Most amphibians are semi-aquatic in that they spend their lives both in the water and on land. Amphibians have thin, permeable skin, which facilitates bioaccumulation. Bioaccumulation can happen over the lifetime of amphibians. It often starts in the egg or larval stage, continues through to adulthood, and can be magnified though consumption of food and water (Donadt et al., 2021).

Frogs are often used in studies of bioaccumulation. Frogs are of particular concern when it comes to the bioaccumulation of toxins in their tissues due to their semi-aquatic lifestyle. Most frog eggs are laid on or in water. The eggs then hatch and enter the larval stage known as tadpoles. Tadpoles live, grow and eat in the water until they develop legs at which time they can also live on land. Adult frogs spend a lot of time in the water which can be a source of Hg accumulation. Another source of Hg contamination in frogs is their diet. Mercury can biomagnify in animals meaning the contaminant can build up within predators and as predators eat contaminated prey, they are exposed to Hg concentrations that are greater than concentrations from direct exposure to the environment (Unrine and Jagoe 2009). This can impact the food chain as more organisms are exposed to contaminants from their prey. For example, frogs may ingest insects that have high Hg concentrations in their tissues and the Hg would be absorbed by the frogs in their digestive tract. Unrine and Jagoe (2009) report that adult frogs will take in less Hg from water as THg in surface waters are typically low, therefore the main source of Hg in frogs is most likely diet (Unrine and Jagoe 2009).

Tadpoles are more susceptible to Hg contamination (Weir et al., 2009). They are aquatic and metals from their environment can be easily absorbed through their skin. While foraging, tadpoles can also ingest sediment which may expose them to chemicals not usually found in the water column. This increases exposure to Hg (Weir et al., 2009). Mercury poisoning can happen in Leopard frog (*Lithobates pipens*) tadpoles if they have MeHg concentrations of over 50 µg/L (Chang et al. 1974). Signs of Hg poisoning in tadpoles include movements that look irritative, difficulty breathing, and swimming abnormally (Weir et al., 2009). Tadpoles are often studied as they are relatively easy to find, they are small, and their life cycle is easily observed.

There are toxicology reports from all over the world that look at the effects of Hg exposure on local amphibian populations. One study by Bank et al., 2009, looked at Hg bioaccumulation in green frog (*Lithobates clamitans*) and bullfrog (*Lithobates catesbeiana*) tadpoles in Acadia National Park in Maine. They found elevated Hg in both species of frog tadpoles. In green frog tadpoles, they found mean total Hg concentrations were 25.1 ± 1.5 ng/g ww. They found the mean total Hg concentrations in bullfrogs to be 19.1 ± 0.8ng/g ww (Bank et al., 2009).

Another toxicology report looked at pig frogs (*Lithobates grylio*) in marshes in the Florida Everglades. This area at the time had health advisories for high content of MeHg concentrations in fish tissues in Water Conservation Areas (WCA) and Big Cypress National Preserve (BCNP) in South Florida (Ugarte et al., 2005). Here they took leg, and other types of muscle tissue samples and looked at THg from 88 frogs. They found the concentration of Hg were highest in the liver and that these concentrations were substantially higher than

concentrations of Hg in leg tissue (Ugarte et al., 2005). This signifies those elevated concentrations of Hg in aquatic pig frog tissues may cause considerable risk to wildlife within some areas in the Florida Everglades (Ugarte et al., 2005).

1.5 Bioaccumulation and Historical Gold Mining in Nova Scotia

Research suggests that amphibians that live in wetlands impacted by gold mine tailings can accumulate Hg and As in their tissues at high concentrations (Yoshino, 2020). Research to date in Nova Scotia, however, has been restricted to either small sample sizes or a limited number of test sites.

While much research has been conducted in terms of assessing As and Hg levels in water, soil, and invertebrates; limited work has been conducted in Nova Scotia assessing the effects of tailings on local amphibian populations. A study by Eaton et al., (1978), sampled sediment, water, and soil samples from 10 abandoned amalgamation sites. Although not the primary research objective, some frogs (n=16) were collected at Montague districts, Oldham districts and Mount Uniacke and were analyzed for concentrations of Hg in frog tissues. A wide range of Hg concentrations was found between the different sites and within the same sites. Concentrations of Hg collected at the Montague (n=4) sites ranged from 0.04 ppm ww to 0.14 ppm ww, at the Oldham (n=5) sites from 0.029 ppm ww to 0.6 ppm ww and at the Mount Uniacke (n=7) sites, from 0.03 pp to 0.53 ppm ww (Eaton, 1978).

At the present time, there is only one other study on the effects of Nova Scotia gold mine tailings on amphibians. Moriarty et al., (2013) assessed the As uptake in green frog (*Lithobates*

clamitans) and eastern American toad (*Anaryxus americanus*). Leg tissue from amphibians caught in Upper Seal Harbour (USH) was analyzed and compared to a reference site. Average concentrations of total As found in frogs at USH (n=7) was 2700 ± 1100 μg kg^{-1}, where at the reference site the average concentration of total As was 230 ± 120 μg kg^{-1}. The total As concentrations at the contaminated site were significantly higher than at the reference site. This study also estimated the daily intake rates of As in frogs by looking at bioaccessibility. Findings showed that the primary source of As exposure was from dermally absorbed water and that ingestion of invertebrates was a secondary source. It was estimated that at the contaminated sites, the daily intake of As is 15-1400 times of that absorbed from the reference sites (Moriarty et al., 2013).

Unique characteristics of the amphibian species may result in amphibians easily accumulating environmental contaminants in their bodies. While As is a significant issue for Nova Scotia, this project focuses on Hg. This research project will test the hypothesis that if concentrations of Hg are high at research sites identified as legacy tailing areas in Nova Scotia, then the concentrations of Hg in the tissues of amphibians collected at the respective research sites will also be high. To test this hypothesis, the concentration of Hg was analyzed from the tissues of amphibians sampled from legacy tailing area research sites and compared to the concentrations found in the tissues of amphibians from a reference site. This project, as part of the legacy gold mine contaminants research led by the Dynamic Environment and Ecosystems Health Research (DEEHR) team at Saint Mary's University, will enhance our understanding of the impact of historical human activity on aquatic ecosystems.

2.0 Methods

2.1 Experimental Design

To determine if concentrations of mercury (Hg) found in amphibian tissues was related to levels of Hg concentrations in their natural wetlands, amphibians were collected from seven research sites within historical gold mining districts in Nova Scotia, Canada. Amphibian sampling started on 13 July 2021 and concluded on 22 September 2021.

2.1.1 Research sites

Research sites are within the Nova Scotia historical gold mining districts of Montague and Waverly (Figure 2). The sites were previously identified as potentially contaminated sites with elevated concentrations of Hg by the Nova Scotia Department of Lands and Forestry. Estimated sediment Hg concentrations, where available, can be found in Table 1. Research was conducted in one reference site, four primary study sites and two supplementary sites. The reference site was used to show baseline contamination conditions in the area. Reference sites can be compared to contaminated sites to illustrate the level of contaminants (ECCC, 2020). The primary sites were identified as the priority sampling sites where all methods of capture would be used. Supplementary sites were identified as sites where sampling would be conducted on an ad hoc basis and be limited to methods that would not involve the installation of sampling apparatus.

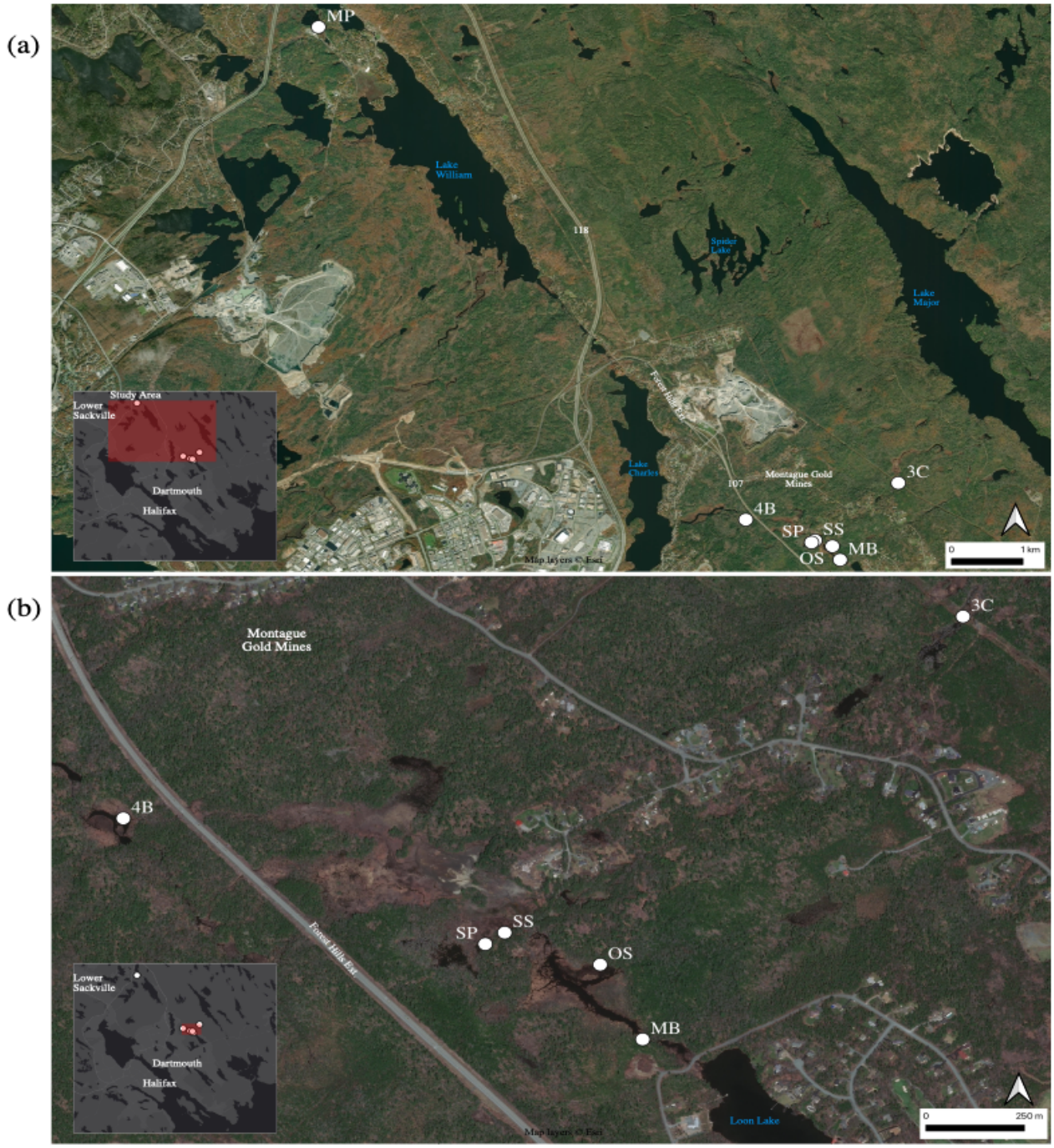

Figure 2. Map of research sites in Nova Scotia (a) Study area. Research sites are located within Montague - 4B, SP, SS, OS, 3C, and Waverly - MP gold mining districts. Reference site was in the Montague district - MB. GPS coordinates can be found in Table 2. Inset map shows overview of Halifax and Dartmouth area. Red box on inset map shows the research area pictured in map (a). (b) Montague research sites. Map showing distribution of research sites in the Montague gold mining district – sites 4B, SP, SS, OS, MB. Inset map shows overview of Halifax and Dartmouth area. Red box in inset map represents area of map (b).

Table 1. Available estimated sediment Hg concentration for research sites.

Research Site	Sediment Hg Concentration (mg/kg)	Source
Reference Site		
Mitchell Brook (MB)	0.39	LeBlanc et al. (2019)
Primary Site		
Barry's Run (4B)	4.27	Intrinsik et al., 2020
Muddy Pond (MP)	20-40	Chapman et al., 2022
Small Pond (SP)	6.2	Parsons et al., 2012
Old Stamp Mill (OS)	24-80	Chapman et al., 2022
Supplementary Site		
Vaughn Lake (3C)	Not available	
Small Stream (SS)	6.2	Parsons et al., 2012

Three of the primary sites are in the Montague district - Barry's Run (4B), Small Pond (SP) and Old Stamp Mill (OS). One primary site is in the Waverly district - Muddy Pond (MP). Supplementary sites are in the Montague district - Vaughn Lake (3C) and Small Stream (SS). The reference site, Mitchell Brook (MB) is in the Montague district. The GPS coordinates for each site have been provided in Table 2.

Table 2. GPS coordinates for each research sites.

Research Site	GPS Coordinate Latitude	GPS Coordinate Longitude
Reference Site		
Mitchell Brook (MB)	44.71117	-63.51616
Primary Site		
Barry's Run (4B)	44.71685	-63.53287
Muddy Pond (MP)	44.78714	-63.60909
Small Pond (SP)	44.71363	-63.52123
Old Stamp Mill (OS)	44.71311	-63.51754
Supplementary Site		
Vaughn Lake (3C)	44.72223	-63.50598
Small Stream (SS)	44.71393	-63.52061

2.1.2 Field work

At each primary site and the reference site, amphibians were collected using four methods of capture (Figure 3). Three of the collection methods involved the installation of apparatus that remained in place throughout the study period - pitfall traps and drift fences, coverboards, and aquatic funnel traps. The fourth method employed a timed search of 30-minutes duration, performed at the beginning of the site visit.

Amphibian collection at the 3C supplementary site was limited to the 30-minute timed search method. At the SS supplementary site, no formal collection method was used. Amphibians were collected if as encountered during site observation.

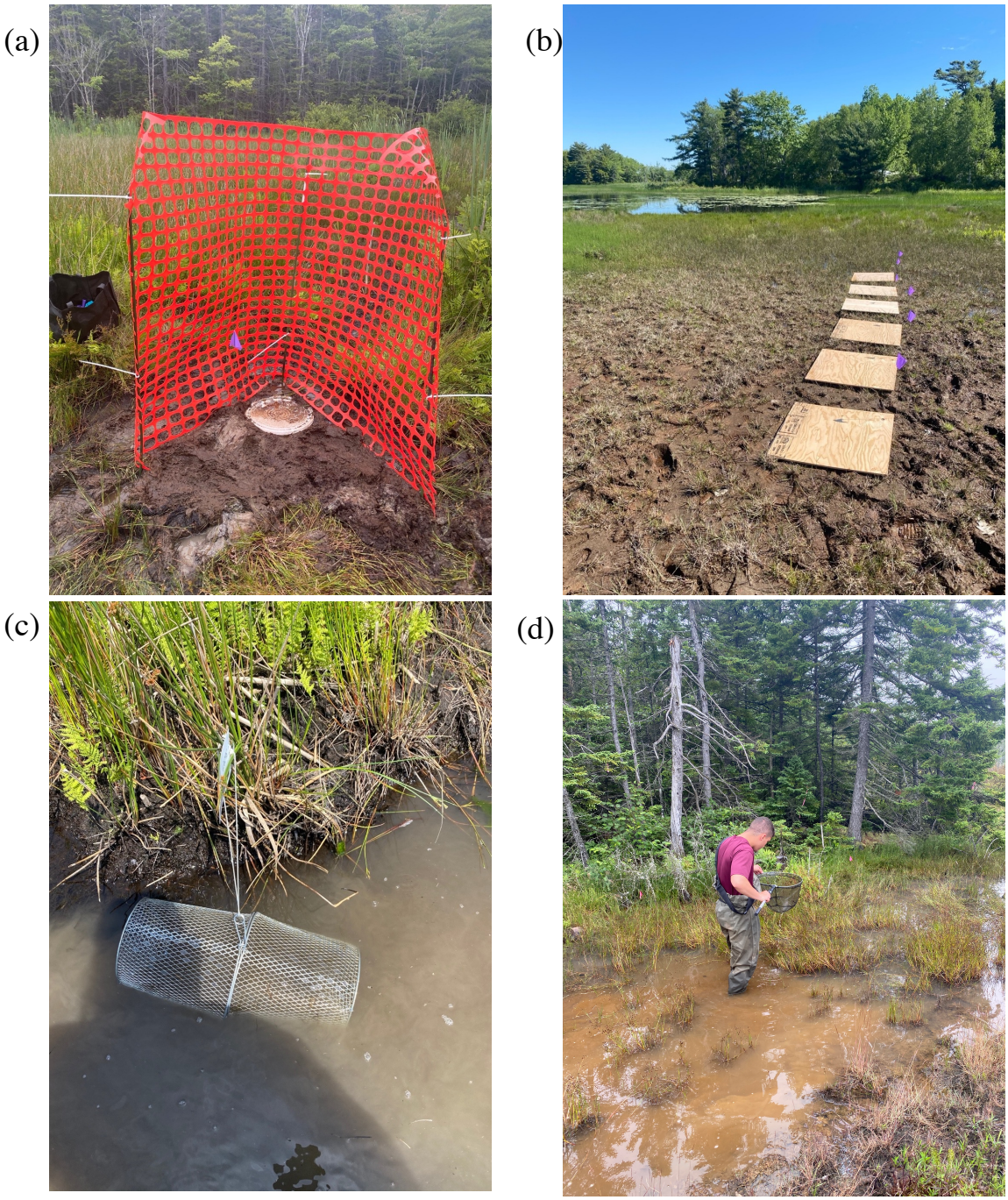

Figure 3. Sampling methods used to collect amphibians during study period. (a) Pitfall trap (bucket) and drift fence (plastic safety fencing) at Barry's Run (4B). (b) Transect of 6 coverboards at Muddy Pond (MP). (c) Aquatic funnel trap placed shallow water at study site 4B. (d) Masters student Catlin Bradbury performing a 30 minute area search using a net at site Old Stamp Mill (OS).

Pitfall traps with drift fences (Figure 3a) are often used in ecological studies to catch small animals. The traps consist of hidden holes dug into the ground that animals can fall into. Drift fences act as barriers placed around pitfall traps and help funnel animals into the traps. Locations for pitfall traps were scouted at each site as part of a sampling plan. Two pitfall traps with drift fences were placed in separate locations at research sites. The locations were close to the waterline of the wetland, while also in a dry and flat area. Pitfall traps were made by digging a hole large enough to accommodate a 5L bucket flush to the ground. Dirt was added to fill in around the bucket. The lid of the bucket was propped open with rocks on one edge to allow a small animal to fall in. Rocks were placed at the bottom of the bucket to weigh it down. As many sites were very wet the buckets would fill with water. This kept captured amphibians moist to avoid desiccation. A bed of moss was placed in the bucket over the rocks to cushion the fall of amphibians. To prevent unnecessary death, traps were not left open for more than 1 day or left over the weekend. To close pitfall traps, the traps were first checked for any animals and the lids of the buckets were snapped on. Drift fences were made using orange plastic safety fencing material cut in 5m lengths. The fencing was held in place using 1.2m long garden stakes and zip ties. Each drift fence was placed around the pitfall trap in a 'V' shape with the pitfall bucket being at the point of the 'V'. The "V" design allowed for the funneling of animals into the trap from the drift fence. During each site visit pitfall traps were checked for amphibian samples by uncovering the traps and propping open the lids. At the end of each visit, the pitfall traps were reset.

As a method of capture, coverboards (Figure 3b) act as artificial places for amphibians to 'hide' or shelter. Coverboards were made from .61m x.61m squares of plywood. Six coverboards were placed in a straight line (transect), starting approximately 5 metres away from the waterline

at each site. Coverboards were labelled 1-6 with small flags. Site visit sampling involved flipping each coverboards and collecting any amphibians found underneath. The coverboards remained in place for the full sampling period (July through September). Amphibians can come and go under coverboards. This sampling approach is beneficial in avoiding desiccation and predation of nocturnal animals during daylight. At the end of each site visit, coverboards were returned to their original position.

At the beginning of each site visit two aquatic funnel traps (Figure 3c) were placed at the waterline of each site near vegetation for capture of tadpoles. At the end of the site visit, the traps were checked for amphibians, and removed from the water.

A timed search of 30 minutes (Figure 3d) was performed at each site at each visit. A timer was set, and the wetland area was actively searched using a D-shaped net. The search area was focused on the waterline surrounding the study wetland and adjacent shallow water areas thick with vegetation. Time used to identify the sample species and prepare samples for transportation was not included in the allotted 30-minute period.

2.1.3 Transportation

At capture, amphibians were placed inside an inflated medium-size Ziploc ® bag (approximately 1L) with wetland water. The bags were labelled with a site code, species code, number code and date that was unique to each amphibian. The bags were place in a small cooler with an ice pack for transportation.

2.1.4 Holding

Amphibians were kept overnight in the Ziploc ® bags. The bags were placed inside a large cooler with ice packs to keep them cool. The cooler was kept in a quiet, cool, dry, room overnight and the amphibians were euthanized in a euthanasia bath the following day.

2.2 Lab work

2.2.1 Euthanasia

A solution of tricaine mesylate, MS-222 (TCI America, Portland, OR, USA) was used to euthanize the amphibians. A stock solution was made by adding 1.5g of MS-222 powder, with 3g of sodium bicarbonate in 25mL of deionized water in a 50mL falcon tube. The tubes of the MS-222 solution were made at the beginning of the project (July 2021) and frozen until needed. When making the euthanasia baths, a tube of the MS-222 solution was defrosted and diluted with 475mL of deionized water. To prevent cross-contamination each site had its own bath. The baths consisted of a small, glass container with a snap-on lid labelled with each site code. When in use the baths were covered with foil to reduce light exposure as MS-222 is known to degrade in daylight.

Once the bath was prepared, one amphibian was placed into the bath at a time. The amphibian was left in the bath for 10 minutes and was then checked for signs of life. Signs of life included movement and response to stimuli. If necessary, time in the bath was extended until the amphibian ceased to show signs of life. The amphibian was then taken out of the bath and placed into a clean dissecting tray ventral side up. The spinal cord was cut with a scalpel to ensure death. The amphibian was then weighed using a portable scale (P-scale, 10205-006, VWR

International, Mississauga, ON) to the nearest ± 0.01g. Body and tibia length were measured using a set of digital calipers (Nicht Wasserdicht, Louisvare Giu Yang, China) to the nearest ± 0.01mm and a photo was taken. Individual amphibians were then returned to original Ziploc ® bags and stored in the freezer.

2.2.2 Amphibian tissue samples

The day prior to dissection, individual amphibians were transferred to a refrigerator to thaw. Once thawed, amphibians in their respective bags were taken out of the fridge and placed on a bed of ice to keep them chilled. One at a time, amphibians were taken out of the Ziploc ® bag and placed on a dissection tray dorsal side up. The limbs were pinned down with dissecting T pins. A scalpel was used to cut the skin of the hind tibia longitudinally from the base of the leg to the patella. Using dissecting scissors, transverse cuts were made below the upper leg and above the patella to free the skin. The skin was moved away from the muscle and pinned. A small section of the muscle tissue was cut and removed using a scalpel and tweezers. The wet weight of the tissue was determined using the same scale as detailed above. The tissue was put in a labelled scintillation vial. The vials were re-frozen in preparation for drying.

As tadpole specimens were too small from which to extract a tissue sample for analysis, they were dried whole. Following euthanasia, a small piece of the tail from each tadpole was removed with a scalpel for possible future identification.

2.2.3 Drying samples

The frozen amphibian tissue samples and whole tadpoles were thawed a day prior to drying. The samples were placed in small tinfoil boats. Samples were dried in a Fisher Scientific drying oven at 55 ℃ for 24 hours. Samples were dried in batches by research site. They were then taken out of their boats and placed in 2.0mL Eppendorf vials and stored in a desiccator until grinding.

2.2.4 Ball Mill Grinder

A Retsch oscillating MM400 ball mill (Retsch USA, Verder Scientific, Inc. Newtown, PA) was used to grind the dried tissue. Two 5mm stainless steel balls were placed inside of the vials which contained the dried tissue sample. The vials were placed in two vial adaptors so that the samples were equally balanced on both sides. The vial adaptors were placed, screwed, and locked into the ball mill. The ball mill was run at a frequency of 30.0 (1/s) for 2 minutes and was repeated until the material was completely ground. The dried, ground tissue was then stored in a desiccator ready for Hg analysis.

2.3 Data Analysis

The dried, ground tissue for each sample was analyzed for total mercury content (THg) using a Direct Mercury Analyzer (DMA-80, Milestone, Sorisole (BG) Italy) in a clean room laboratory at Saint Mary's University. Clean quartz boats were used to run samples. To ensure no cross-contamination, trace-element protocols were followed. To ensure quality control, each run of the DMA-80 analyzer started with multiple blanks, followed by series of Hg standards (0, 5, 10, and 20 ppm), as well as three certified reference materials (CRM) - Tort 3, Dorm4, and

Dolt5 - to check machine calibration. For quality assurance, replicates were run every 20 samples. To prevent contamination carry-over of samples, two to four blanks were run between research sites and between every 20 samples. Total mercury content data was recorded as mg/kg. Results were categorized by amphibian life – either adult or tadpole.

2.3.1 Statistical Analysis

Statistical Analysis was performed using RStudio Version 4.1.1. A Levene's test of equality of variances was performed. Differences in THg at each site was determined using a one-way analysis of variance (ANOVA). To compare means of study sites, post-hoc Tukey tests were performed. Differences were considered significant at $P < 0.05$.

3.0 Results

3.1 Amphibian Samples Collected

Amphibian samples collected at research sites and used in this study included adult frogs, and toads and tadpoles (Table 3). Data for adult amphibians includes all species of adult amphibians found during the sampling period. The majority of frogs were caught during a timed search of 30 minutes (n=36). Adult amphibian species caught per site are shown in a bar graph (Figure 4). Three species of adult amphibians were collected - Green frog (*Lithobates clamitans*) (n=35), Leopard frog (*Lithobates pipiens*) (n=1), and Pickerel frog (*Lithobates paulstrisis*) (n=1). The Eastern American toad (*Anaxyrus americanus*) (n=2) was also found. Data for the adult toads is pooled with adult frog data. Two species of tadpoles were collected - Green frog (n =

27), and Mink frog (*Lithobates septentrionalis*) (n=2). Tadpole species caught per site are represented in a bar graph (Figure 5).

Table 3. Number of amphibian samples collected at respective research sites.

Research Site	# **Adult Frogs**	# **Toads**	# **Tadpoles**
Reference Site			
Mitchell Brook (MB)	7	0	9
Primary Site			
Barry's Run (4B)	3	0	7
Muddy Pond (MP)	3	1	0
Small Pond (SP)	4	0	0
Old Stamp Mill (OS)	7	1	2
Supplementary Site			
Vaughn Lake (3C)	11	0	10
Small Stream (SS)	2	0	1

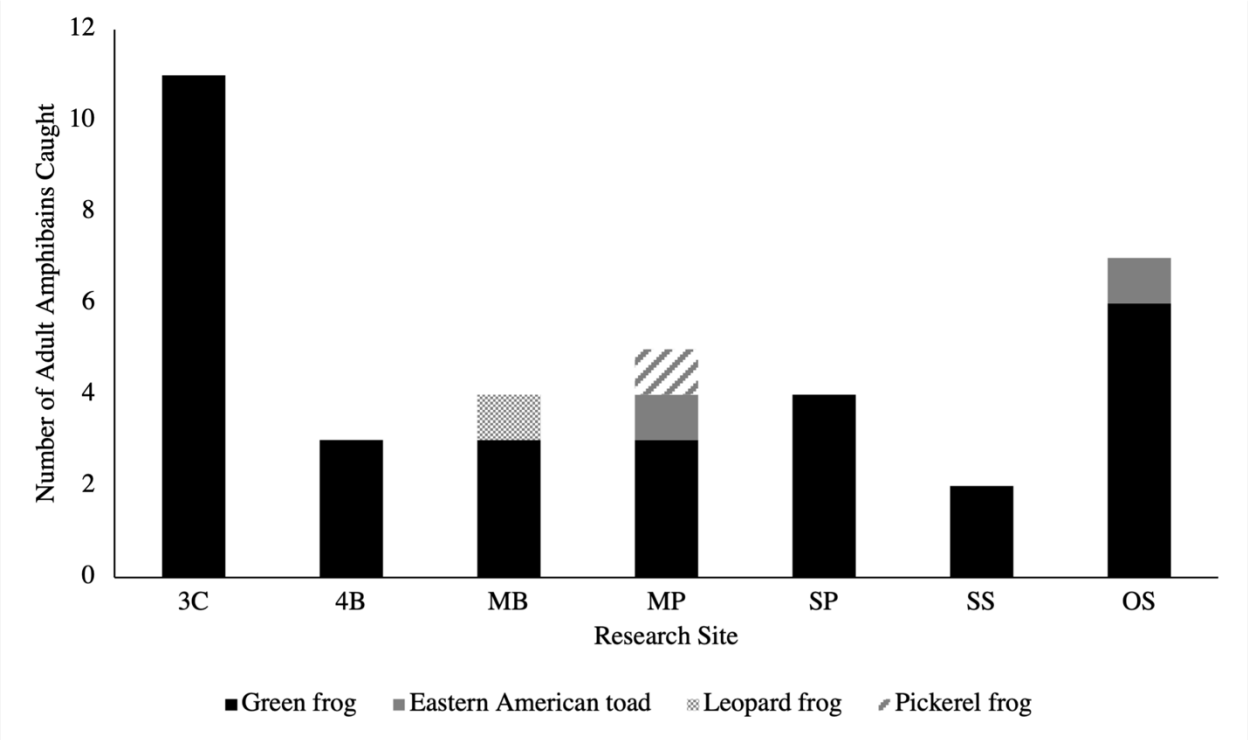

Figure 4. Bar graph representing the number of individual adult amphibian species collected at each research site. Individual species identified included Green frog (*Lithobates clamitans*), Leopard frog (*Lithobates pipiens*) Pickerel frog (Lithobates paulstrisis) and the Eastern American toad (*Anaxyrus americanus*).

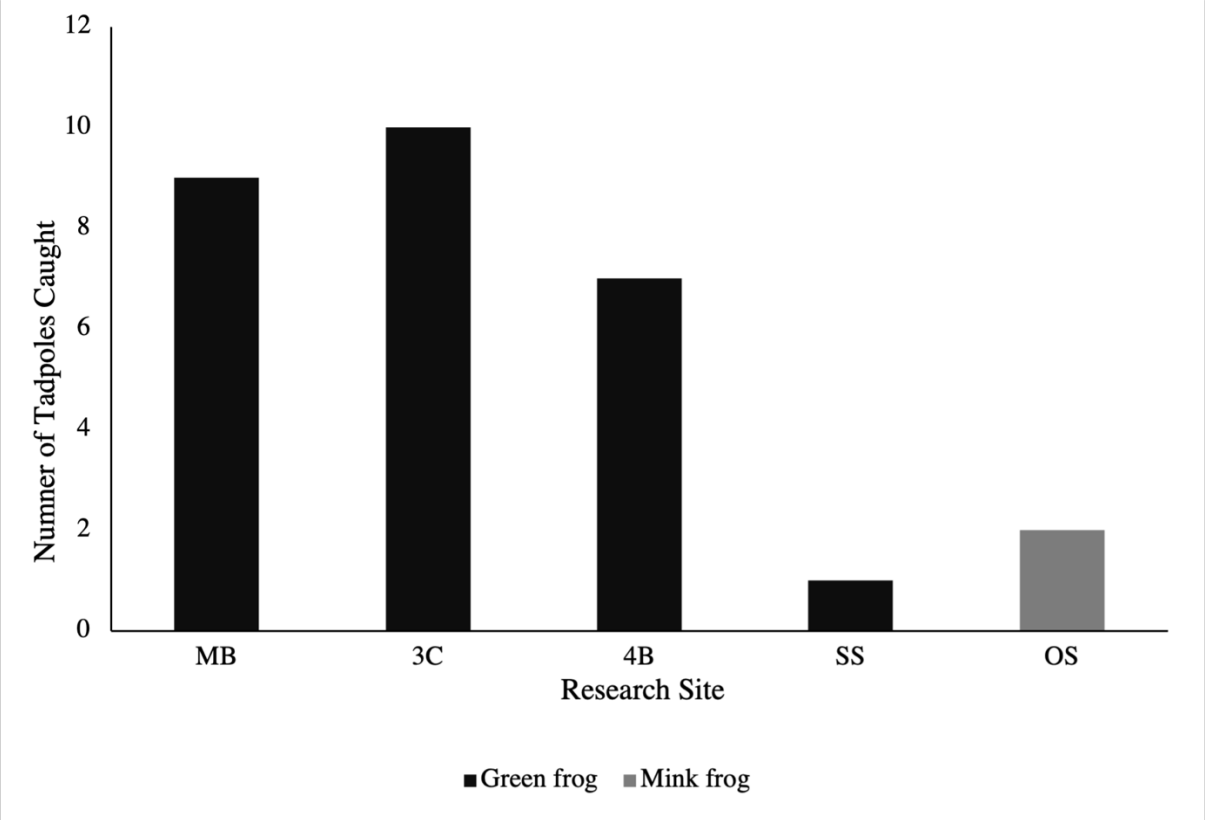

Figure 5. Bar graph representing the number of tadpoles of each species collected by research site. Species identified were Green frog (*Lithobates clamitans*), and Mink frog (*Lithobates septentrionalis*).

3.2 Adult Amphibians

Mean THg concentrations were calculated and compared among sites for adult amphibians using a one-way analysis of variances (ANOVA). Results are presented in a box plot (Figure 6). The results of the Tukey test show that the frogs from Small Stream (SS) had significantly different concentrations of THg when compared to Muddy Pond (MP). At SS the concentrations were found to be significantly higher than those at MP. No other sites were found to be significantly different when compared to each other.

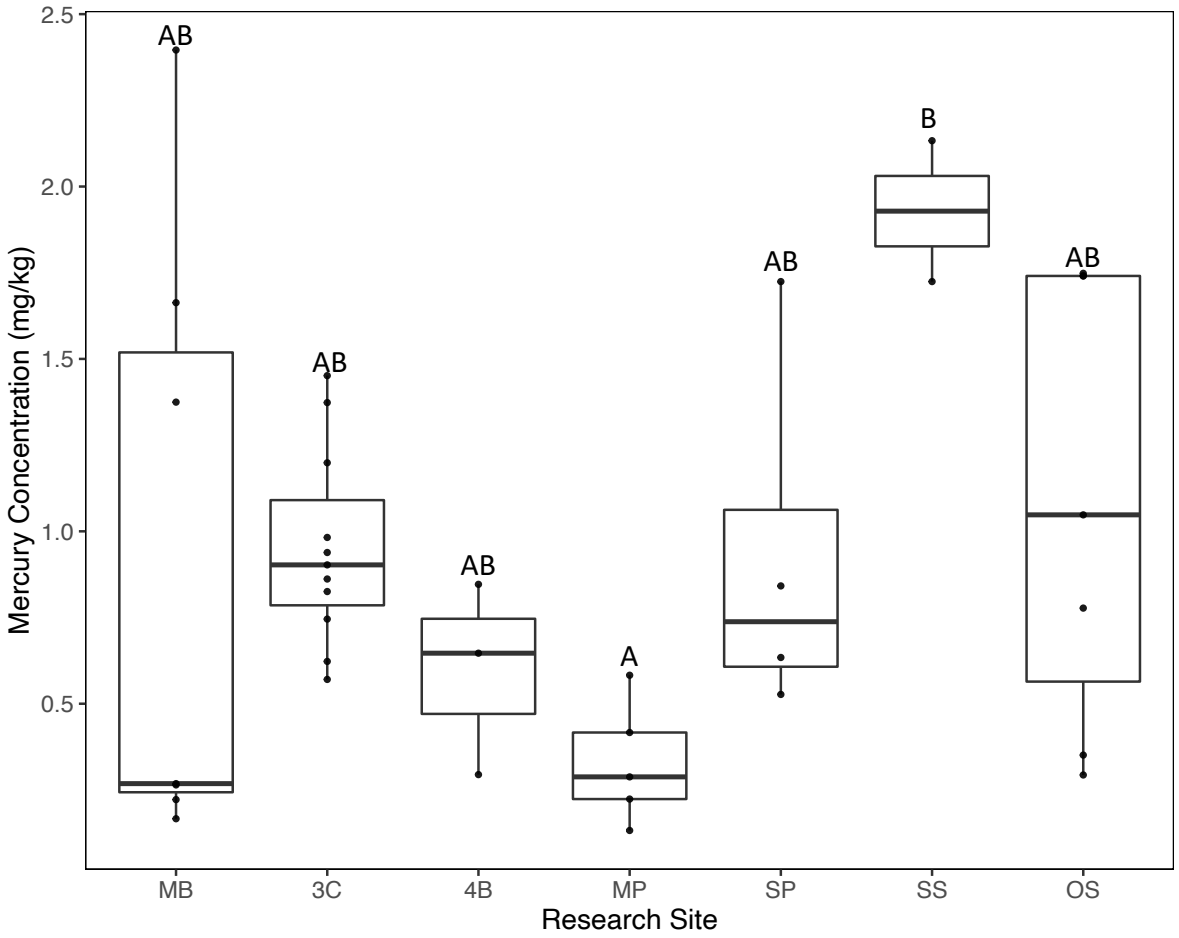

Figure 6. Box plot of THg concentrations in frog tibia muscle (n=39) measured in mg/kg at each study site. Horizontal bars indicate the median, boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range. Mean values are provided in Table 4. The ANOVA indicates that sites identified with different letters (A,B) are statistically different.

Mean THg concentrations for adult frogs are presented in Table 4. A range of THg concentrations was observed in adult amphibians from 0.1324 – 2.3959 mg/kg. Small Stream (SS) was found to have the highest mean with THg concentrations ranging from 1.7243 – 2.1329 mg/kg followed by Old Stamp Mill (OS), Vaughan Lake (3C), Small Pond (SP), Mitchell Brook (MB) and Barry's Run (4B). The one research site in the Waverly district, MP had the lowest mean with THg concentrations ranging from 0.1324 - 0.5826 mg/kg.

Table 4. Mean THg concentration, and min and max values in adult amphibians at historical gold mine tailing areas and reference site.

Research Site	Sample Size (n)	Mean THg Concentration (mg/kg)	Minimum	Maximum
Reference Site				
Mitchell Brook (MB)	7	0.9078± 0.8987	0.1664	2.3959
Primary Site				
Barry's Run (4B)	3	0.5957 ± 0.2794	0.2944	0.8462
Muddy Pond (MP)	4	0.3285 ± 0.1757	0.1324	0.5826
Small Pond (SP)	4	0.9317 ± 0.5443	0.5269	1.7243
Old Stamp Mill (OS)	8	1.0999 ± 0.6532	0.2933	1.7482
Supplementary Site				
Vaughn Lake (3C)	11	0.9521 ± 0.2850	0.5705	1.3733
Small Stream (SS)	2	1.9286 ± 0.2888	1.7243	2.1329

3.3 Tadpoles

To look at significance of Hg concentrations in tadpoles mean THg concentrations were calculated for each site and compared (Figure 7). The SS site was excluded from the analysis as only one tadpole was collected from this site. Using an ANOVA test, OS was found to have significantly higher concentrations of THg when compared to all the other sites where tadpoles where collected – MB, 3C and 4B. Mitchell Brook (MB), 3C and 4B were found to be not significantly different from each other.

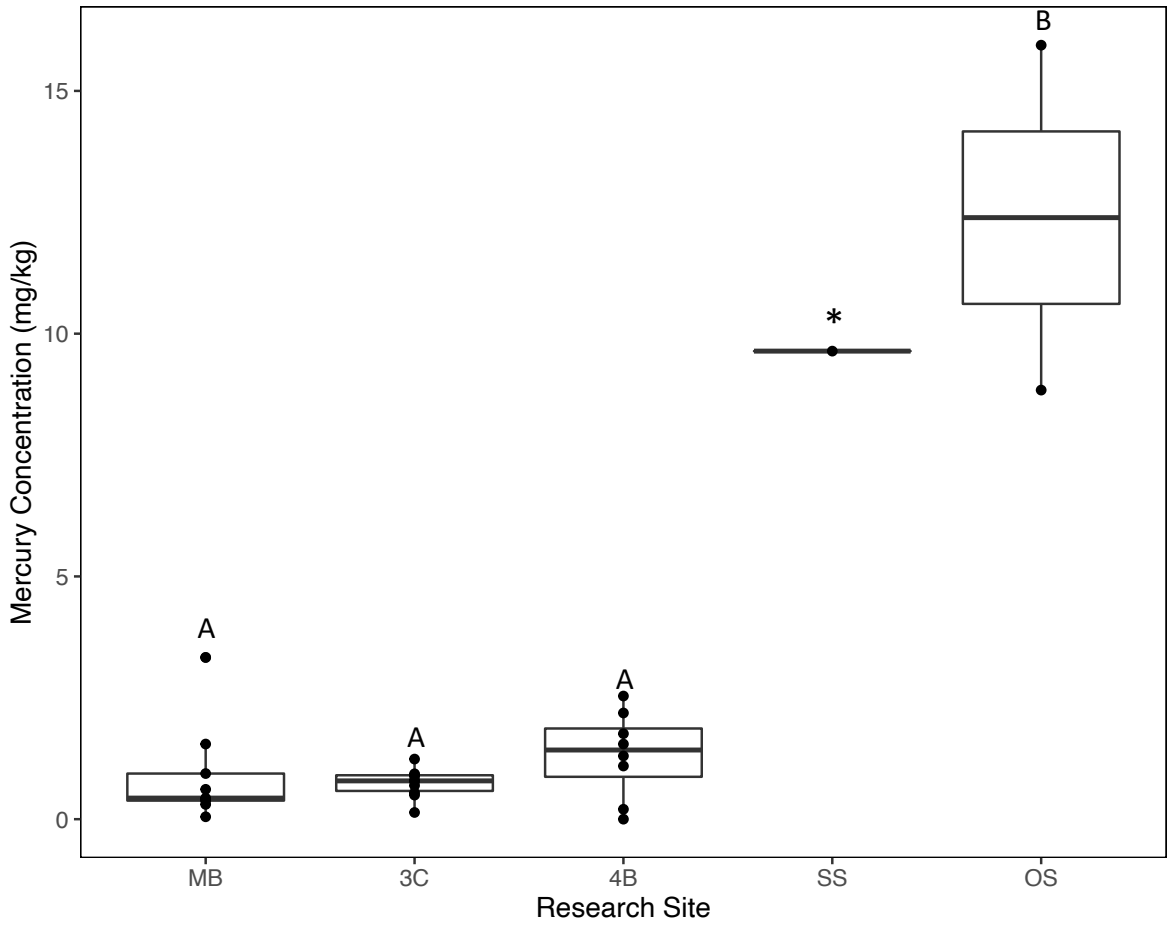

Figure 7. Box plot summarizing THg concentrations in whole tadpole samples (n=29) measured in mg/kg for all sites. Horizontal bars indicate the median, boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range. Mean THg concentration values are provided in Table 5. The ANOVA indicates that sites identified with different letters (A,B) are statistically different. The SS site was excluded from the ANOVA (indicated by the *) due to the low sample size.

Mean THg concentrations for tadpoles are presented in Table 5. A large range of THg concentrations was observed in tadpoles from 0.0494 – 15.9412 mg/kg. Old Stamp Mill (OS) was found to have the highest mean with THg concentrations ranging from 8.8372 – 15.9412 mg/kg. Total mercury content concentrations at MB, 3C and 4B were found to be similar. Tadpoles with the lowest concentrations of THg were found at the reference site MB. Vaughn Lake (3C) however was found to have the lowest mean concentration of THg

Table 5. Mean THg concentration, and min and max values in tadpoles at historical gold mine tailing areas and reference site.

Research Site	Sample Size (n)	Mean THg Concentration (mg/kg)	Minimum	Maximum
Reference Site				
Mitchell Brook (MB)	9	0.8905 ± 1.013	0.0494	3.3312
Primary Sites				
Barry's Run (4B)	7	1.519 ± 0.7634	0.2043	2.5364
Old Stamp Mill (OS)	2	12.2396 ± 5.0236	8.8372	15.9412
Supplementary Sites				
Vaughn Lake (3C)	10	0.7421 ± 0.2988	0.1384	1.2380
Small Stream (SS)	1	9.6405	N/A	N/A

4.0 Discussion

Animals such as amphibians have been used in many research studies looking at bioaccumulation (Bank et al., 2009, Boczulak et al., 2017, Unrine and Jagoe, 2009). Legacy gold mining has left some wetland areas in Nova Scotia with very high concentrations of contaminants (Parsons et al. 2012). Although wetland areas studied within this project are well known to be contaminated from legacy gold mining, limited work has been done which focusses on the impact of contaminants, such as Hg, on the local amphibian population. As amphibians are prey for wildlife such as birds, raccoons, foxes and snakes, predators up the food web may be impacted by these contaminants. This study found elevated concentrations of THg in both adult amphibians and tadpoles at Nova Scotia wetland research sites.

4.1 Adult Amphibians

Based on previous studies in the area, as well as tailing maps, the research site identified as Old Stamp Mill (OS) was expected to have the highest concentrations of Hg in amphibians followed by Muddy Pond (MP), Small Pond (SP), Small Stream (SS), Barry's Run (4B), Vaughn Lake (3C) and Mitchell Brook (MB) (Chapman et al, 2022, Parsons et al., 2012, Intrinsik et al., 2020, LeBlanc et al., 2019).

Although tissue analysis showed that adult amphibians from the OS site had the highest mean concentrations of THg, ANOVA showed that there were no significant differences in tissue THg concentrations among sites. Only two sites were found to have significantly different concentrations of THg – SS and MP. The reference site, MB had higher tissue concentrations of THg than expected. Concentrations in adult amphibians ranged from 0.1324 - 2.3959 mg/kg. These numbers are slightly higher than previously reported Hg concentrations in the area. Eaton (1978) reported Hg concentrations in frog tissues to be 0.04 – 0.14 mg/kg.

This project used MB as a reference site. MB was chosen as it has been used in many previous studies as a reference area for the Montague historical gold mining district. Previous studies examined sediment, water, and small invertebrates (Parsons et al., 2012, Chapman et al., 2022, LeBlanc et al., 2019). All were found to have significantly lower concentrations of Hg when compared to tailing sites in the same area. Reference areas are used as they reflect physical and chemical conditions, such as climate, substrate, and habitat, without the stressors or risks of concern within the contaminated area (ECCC, 2019). Reference sites provide a baseline for comparison of contaminant concentrations which supports the identification of sites where

concentrations may be elevated. While MB has been deemed appropriate for sampling water, sediment, and small invertebrates it does not appear to be an appropriate reference site for adult amphibians.

Past research has determined the home ranges of frogs. Home range refers to the area in which an animal could normally travel to get food. (Martof 1953). A map of the research sites used, with the radius of the home ranges for frogs is presented in Figure 8. Green frogs tend to have a large variation in home ranges. The minimum range was reported to be 20sq m, maximum range was found to be 200sq m and the average home range of frogs was reported to be 61sq m (Martof 1953). Due to the fact that frogs have large and varied home ranges, it is important that reference sites are far enough away from the contaminated research sites to ensure that frogs caught at the contaminated sites do not travel to the reference site and vice versa. Looking at the maximum radius of home range in green frogs, there is a large overlap in the sites OS and MB. The proximity of the two sites may support back and forth travel by frogs which may result in an inaccurate representation of the level of contamination at the reference site. This could account for the slightly elevated concentrations of THg seen in adult amphibians caught at reference site MB. As sites OS and MB are located in a small area with varying concentrations of contaminants, using MB as a reference site is not recommended to study concentrations of contaminates in adult amphibians.

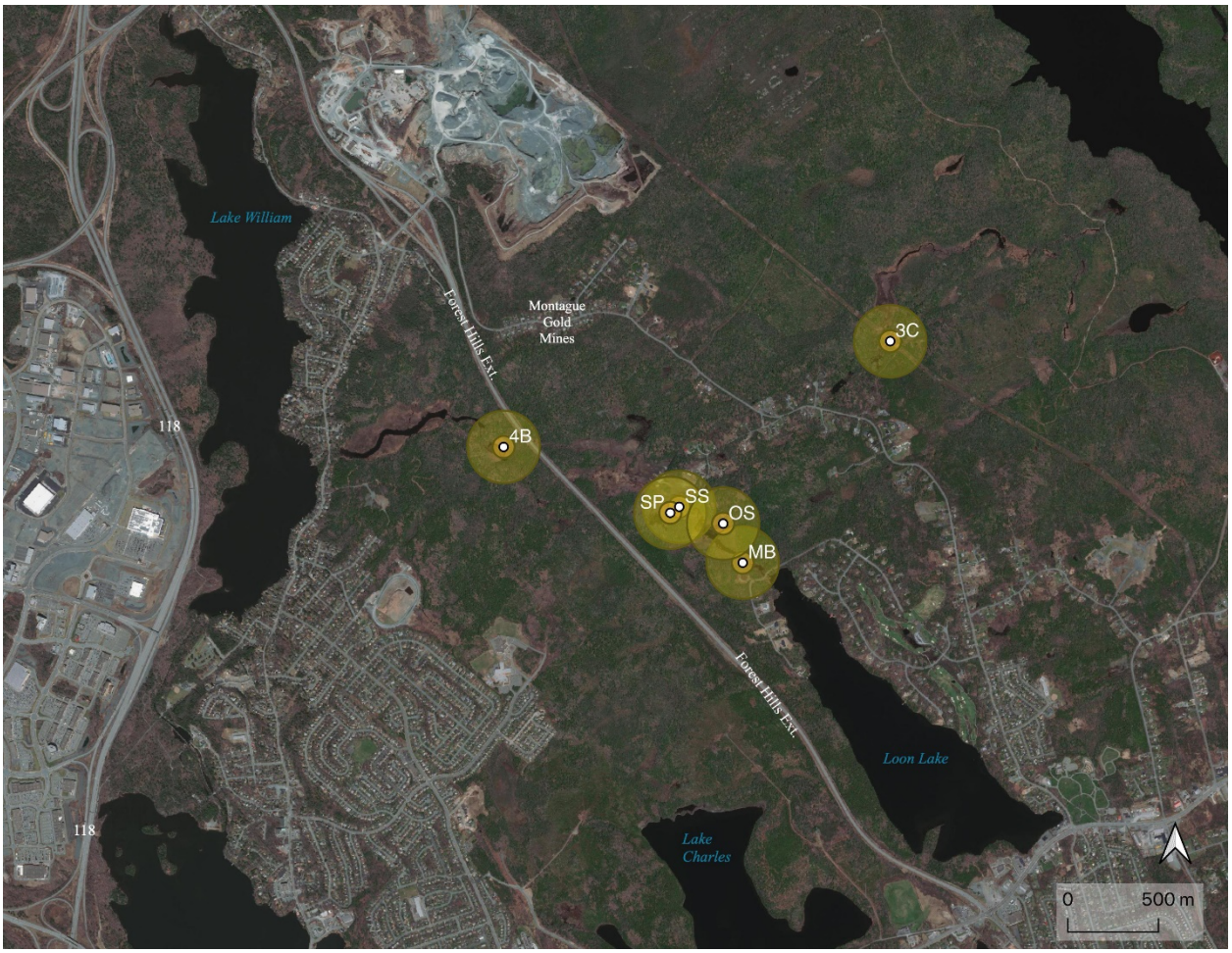

Figure 8. Map of research sites with radius of home ranges of green frogs. Each site is represented by a white point and labeled. The mean radius (61 sq m) of potential frog home range is represented by the inner, darker yellow circle, the maximum potential frog home range radius (200 sq m) is represented by the larger, light-yellow circle.

4.2 Tadpoles

Concentrations of THg in tadpoles were found to reflect the patterns of concentrations seen in their environments. Results show that the tadpoles with the highest concentrations of THg were found at OS, the research site with the highest sediment Hg. Tadpoles with some of the lowest concentrations were found at the reference site MB. The results of the tadpole analysis also reflected this relationship as OS was found to have significantly higher THg concentrations when compared with all other sites where tadpoles were collected – MB, 3C, and 4B. Tadpole

results have THg concentrations that range from 0.0494 - 15.941 mg/kg. These values are higher than previously reported values for green frog tadpoles presented in Table 6 (Eaton 1978, Bank et al. 2009, Weir et al. 2009, Boczulak et al. 2017). A study was done by Weir et al., (2009) that looked at green frog (*Lithobates clamitans*) and bullfrog (*Lithobates catesbiana*) tadpoles. These tadpoles were collected at four coal-fired power plants and 29 wetlands in southern Illinois. The concentrations of Hg for homogenized green frog tadpoles were found to range from 0.005 to 0.157 mg/kg with a mean of 0.0465 ± 0.0059 mg/kg (Weir et al., 2009). Mercury in bullfrog homogenized tadpoles ranged from 16 to 197 ng/g ww with a mean of 56.8 ± 6.6 ng/g ww (Weir et al., 2009).

Total mercury concentration ranges in tadpoles (0.0494 - 15.941 mg/kg) found in this study are more in line with Hg concentrations in a study done with Wood frog (*Lithobates sylvaticus*) tadpoles and Boreal Chorus frog (*Pseudacris maculate*) tadpoles found in the Prairie Pothole region of Canada (Boczulak et al., 2017). In the Bozulak et al. (2017) study, Hg concentrations in wood frog tadpoles ranged from 0.0259 - 7.8893 mg/kg and in chorus frog tadpoles from 0.0005 – 2.3000 mg/kg. While these literature values are similar to concentrations of THg found at research sites MB, 3C and 4B, the THg concentrations found in tadpoles at research sites OS and SS were even more elevated.

Not much work has been done to identify home ranges for tadpoles. This concept may not applicable as, at this stage of life, tadpoles are strictly aquatic and are not exposed to water outside their self-contained environment. Tadpoles mainly accumulate contaminants through their skin from the water in their environments, in their diets, and from the sediment they pick up

while foraging (Weir et al., 2009). As a result, the study of THg concentrations in tadpoles may provide a more accurate picture of contaminant levels in the immediate environment, in particular water and sediment, as compared to the study of adult frogs, who often are exposed to contaminants though their diets.

Table 6. Literature values for Hg concentration in frogs and tadpoles. Units were converted to mg/kg and ww converted to dw assuming 83% moisture for tadpoles.

Species	Life stage, type of sample	Location	Type of site	Range of Hg Concentration (mg/kg), min-max (N)	Source
Green frog (*Lithobates clamitans*)	Tadpole homogenized	Southern Illinois, US - reference site	Contaminated (Coal-Burning emissions)	0.005 – 0.157	Weir et al., 2009
Bull frog (*Lithobates catesbiana*)	Tadpole homogenized	Southern Illinois, US - reference site	Contaminated (Coal-Burning emissions)	0.013- 0.0318	Weir et al., 2009
Green frog (*Lithobates clamitans*)	Tadpole, whole	Maine, US	Contaminated (Acadia National Park)	0.0251 ± 0.015	Bank et al., 2009
Bull frog (*Lithobates catesbiana*)	Tadpole, whole	Maine, US	Contaminated (National Park)	0.0191 ± $8.0x10^{-4}$	Bank et al., 2009
Unidentified frog (no species name given)	Frog leg tissue	Montague Gold Mine District (legacy gold mine tailing sites)	Legacy gold mine tailings	0.04 – 0.14	Eaton, 1978
Boreal Chorus frog (*Pseudacris maculate*)	Tadpole, whole	Prairie Pothole Region, CA	Contaminated (Wetland Ponds)	0.0005 – 2.3000 (n=32)	Boczulak et al., 2017
Wood frog (*Lithobates sylvaticus*)	Tadpole, whole	Prairie Pothole Region, CA	Contaminated (Wetland Ponds)	0.0259-7.8893 (n=61)	Boczulak et al., 2017

The low concentration of THg found in tadpoles at MB appears to reflect the expected low concentration of contaminants at the reference site. This coupled with the restriction of tadpoles to small, local, and self-contained water bodies suggests that MB was a more suitable

reference site for tadpoles than for mobile, post-metamorphic frogs which may disperse and pick up contaminants across MB and into OS.

Sampling took place between mid-July and late September. This was the breeding period for the green frog, which is likely why the majority of samples caught were green frogs. Most species of Nova Scotia frogs and toads start becoming more active between the months of April and June. Ideally this would have been the optimum time to begin sampling as the increase in frog activity may have resulted in a larger and more diverse sampling of amphibians. COVID-19 travel protocols together with COVID-19 impacts on administrative processes such as submitting necessary animal care forms and obtaining required permits resulted in a sampling start date that was later than ideal. If sampling were to have started earlier, Wood frogs (*Lithobates sylvaticus*), Spring Peeper frogs (*Pseudacris crucifer)*, and possibly some species of salamanders may have been found. An earlier start date is recommended for future studies to optimize the potential number and diversity of amphibian samples.

In conclusion, this study found that the concentrations of THg in adult amphibians sampled from research sites in historical gold mining areas do not reflect the patterns Hg concentrations in their environments. This study further found that the concentrations of THg in tadpoles sampled from research sites in historical gold mining areas do reflect the patterns of Hg concentrations in their environments. It is recommended that tadpoles, as opposed to adult amphibians, be used for the study of bioaccumulation of contaminants in amphibians where research study sites are in close proximity and have highly varied concentrations of contaminants.

It is important to note that adult amphibians with elevated concentrations of THg were found at the contaminated research sites and, as an important part of their ecosystem, they are potentially transferring Hg within and between sites which may pose health risks to their predators.

Based on a review of the literature, most studies looking at Hg contamination in amphibians have focused on tadpoles. Future research, with careful consideration of factors such as sampling period, site proximity and contaminant concentrations, could provide valuable insight into how contaminants, such as Hg, can bioaccumulate in amphibians throughout their lifecycle.

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