

**Bioaccumulation and Transfer of Mercury and Arsenic in Aquatic Invertebrates
and Emergent Insects at Historical Gold Mine Tailing Sites of Nova Scotia**

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ABSTRACT

Historical gold mining in Nova Scotia resulted in over 3,000,000 tonnes of mine tailings deposited into aquatic habitats and low-lying areas, where they remain today. Legacy tailings are typically elevated in mercury (Hg) and arsenic (As), however, aquatic ecological effects remain largely unquantified to date. An initial literature review revealed only three studies mentioning contaminants in aquatic invertebrates at tailing sites. My objective was to assess [Hg] and [As] in aquatic invertebrates living on tailings-affected wetlands, and the role of emergent insects as biovectors of these contaminants. Samples showed that sediment and water at tailings sites were elevated in Hg and As, often surpassing CCME guidelines. Aquatic invertebrates from tailings sites had elevated [Hg] (up to 4.20 ppm). Invertebrate [As] frequently exceeded CCME guidelines for fish. Adult emergent insects were shown to be likely biovectors of Hg, while As was largely shed with casings during hatching.

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*To Uncle Anthony –
Thank you for sharing your love of learning.*

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Chapter 1. Review of ecological mercury and arsenic bioaccumulation in historical gold mine districts of Nova Scotia

ABSTRACT

Gold mining in Nova Scotia dates back to the mid 1800s. Historical industrial practices generated over 3,000,000 tonnes of finely-ground mine wastes (tailings) which were deposited into lakes, streams, wetlands and low-lying areas close to the mill sites. These legacy tailings are typically contaminated with arsenic (As) and mercury (Hg) and continue to impact downstream environments to this day. The objective of this review is to critically examine and summarize existing knowledge on transfer and bioaccumulation of Hg and As in aquatic and terrestrial organisms exposed to legacy gold mine tailings in Nova Scotia. This review reveals that 24 previous studies have been completed on this subject. A number of these studies were based on small sample sizes, or had other limitations, such as missing proper identification of species. Despite these limitations, the data presented in these publications clearly indicate that both Hg and As from the abandoned gold mine sites are bioaccumulating in plants, fungi, freshwater and terrestrial invertebrates, marine mollusks, amphibians, fish and mammals. In many cases, concentrations of Hg and As in tissue exceed Canadian Council of Ministers of the Environment (CCME) safety guideline values for wildlife consumption. No studies were found examining tailings-related Hg or As accumulation in lichen, reptiles, or large mammals. This review concludes that further research on bioaccumulation and biomagnification of tailings-related Hg and As is needed to understand the overall impact of historical tailings on the aquatic and terrestrial ecosystems and species of Nova Scotia.

More detailed studies are vital for guiding risk-management, decision making and land-use practices for these contaminated sites.

INTRODUCTION

Gold mining has been an important part of Nova Scotia's history and culture since the mid-1800s. There are 64 formal gold mining districts containing hundreds of individual mines, stretching across mainland Nova Scotia (Bates, 1987; Art Gallery of Nova Scotia, 2013). The initial discovery of bedrock gold mineralization occurred near Mooseland in 1858 (Malcolm, 1929). In the decades that followed, the gold industry became an integral part of Nova Scotia's economy and played a major role in the development of many small mining towns. Between 1861 and the mid-1940s, there were three distinct gold rushes resulting in the production of an estimated 1.2 million troy ounces (approximately 37,000 kg) of gold (Bates, 1987). This is likely a conservative estimate, as historical mining companies commonly underestimated the amount of recovered gold to avoid paying royalties to the government (Art Gallery of Nova Scotia, 2013).

During this era of gold mining, most auriferous (gold containing) ore in Nova Scotia was crushed to sand or silt-sized material using stamp mills or ball mills, and then the gold extracted using Hg amalgamation techniques (Blakeman, 1978). Fresh water from nearby aquatic ecosystems was used to wash the crushed ore over Hg-coated copper plates. Gold particulates formed an amalgam with the Hg, which was scraped from the plates and heated to boil off Hg, thus leaving the remaining gold. It has been estimated that one ounce of Hg was used for every ounce of gold recovered, although higher

estimates of Hg per ounce of gold have been reported (Parsons et al., 2012). Beginning in the 1890s, gravity separation, roasting, chlorination, and cyanidation were also added to the milling circuit at some mines to recover gold from sulphide minerals and older tailings, but Hg amalgamation remained an essential part of most of these mills until the 1940s.

Residual sand- to silt-sized waste material (also called tailings) was commonly deposited into nearby lakes, streams or wetland ecosystems, after the gold was extracted. There are currently an estimated 3,000,000 tonnes of historical gold mine tailings throughout the province (Parsons et al., 2012). Although most Nova Scotian gold mining districts are now long-abandoned, a legacy of significant environmental contamination at the historical stamp mill sites and tailings disposal areas remain today (Parsons et al. 2012; Drage, 2015). The Hg recovery process during the late 1800s to early 1900s was inefficient, and an estimated 10-25% of the Hg used was lost to tailings or to the atmosphere during this time (Eaton, 1978; Parsons & Percival, 2005).

Mercury is not an essential element required for biological functioning, and many forms of Hg can be highly toxic, including dimethylmercury, methylmercury (MeHg) and elemental mercury (Hg^0). Hg^0 was the form used for gold amalgamation at historical gold mines in Nova Scotia, and is still used in artisanal gold mines in some countries. It is a silvery metal, liquid at room temperature and highly volatile. When Hg^0 enters into an aquatic environment, either from mining wastes or atmospheric deposition, it may oxidize, forming inorganic Hg (Hg^{II}), which has a much higher solubility in water (O'Driscoll et al., 2005). When Hg^{II} is present in acidic environments with anaerobic conditions, high microbial activity, and high levels of organic carbon (i.e. many wetland

conditions), it may undergo methylation by microorganisms such as sulphate-reducing (SRB) and ferric-reducing bacteria (FeRB), which can produce organic Hg compounds, including MeHg (O'Driscoll et al., 2005).

Methylmercury is especially toxic to living organisms, highly bioavailable, and lipophilic, allowing it to pass through membranes, including the blood-brain barrier and the placental barrier (Park & Zheng, 2012). In this way, MeHg can reach vital organs and accumulate in the brain (CCME, 2003; Edmonds et al., 2010; Doe et al., 2017).

Additionally, MeHg is eliminated from the body very slowly, making it more likely to bioaccumulate and biomagnify in higher predators (O'Driscoll et al., 2005). It has been shown to account for the majority of total Hg (THg) accumulation in aquatic organisms (Lavoie et al., 2013).

Along with Hg, tailings also typically have elevated As concentrations (Parsons et al. 2012). Most gold is hosted by quartz veins within meta-sandstones and slate of the Cambro-Ordovician Meguma Supergroup, which makes up most of the southern mainland of Nova Scotia. Arsenopyrite (FeAsS) is the most commonly occurring sulphide mineral in these auriferous veins and surrounding host rocks and contains 46 wt.% As by mass (Kontak & Jackson, 1999). When arsenopyrite in tailings and other mine waste is exposed to the atmosphere, it slowly weathers and oxidizes, releasing As into the surrounding environment (Lengke et al., 2009).

Arsenic is a metalloid with highly complex chemistry and over fifty naturally occurring species identified, each with varying toxicities to humans and other organisms (Cullen & Reimer, 1989; Francesconi & Kuehnelt, 2004). The toxicity of As depends greatly on its speciation, bioavailability, concentration and the detoxification mechanisms

of exposed organisms (Cullen & Reimer, 1989). In general, contrary to Hg, inorganic As (IAs) forms are more toxic than organic forms, as they are proven carcinogens (Mass et al., 2001; Ng, 2005). It is these inorganic species, including arsenite (As^{III}) and arsenate (As^{V}), which often enter into freshwater systems following erosion of mine tailings (Campbell and Nordstrom, 2014). Microbial reactions may also release As from the solid to aqueous phase, increasing the mobility of As in freshwater environments (Oremland & Stolz, 2003).

Arsenic has been shown to accumulate in biota through their contact with, and ingestion of, contaminated water, sediment, soil and/or organic matter, but despite numerous studies, there remains much uncertainty around the biomagnification potential of As (Rahman & Hasegawa, 2012). Within living organisms, As is mainly found in organic forms, such as arsenobetaine (often found in marine organisms) and arsenocholine, both of which are relatively non-toxic (Cullen & Reimer, 1989). However, more toxic inorganic species have been found to bioaccumulate in both terrestrial and aquatic organisms, including in a number of species within Nova Scotia including plants (Koch et al., 2007; Saunders et al., 2011), invertebrates (Chapman et al., 2016; Moriarty et al., 2009), shellfish (Koch, 2007, Whaley-Martin et al., 2012, 2013, Walker and Grant, 2015), amphibians (Moriarty et al., 2013, Saunders et al., 2010, 2011), and mammals (Moriarty et al., 2011, Saunders et al., 2010, 2011) among others (Rahman & Hasegawa, 2012).

The first environmental studies of gold mine tailings in Nova Scotia took place in the late 1970s, after a resident living near a historical gold district (Waverley) was diagnosed with chronic As intoxication following exposure to contaminated well water

(Hindmarsh et al., 1977). Several studies over the subsequent four decades have shown that tailings from historical gold mines have high concentrations of both As and Hg and, at some sites, have contaminated downstream environments (e.g. Eaton, 1978; Eaton & Clair, 1985; Wong et al., 1999; Koch et al., 2007; Parsons et al., 2012;). From 2003 to 2012, detailed studies by Natural Resources Canada, several universities, and other government departments helped to characterize the environmental and human health hazards associated with 14 of these historical gold mine districts and provided guidance for site management and remediation (Parsons et al., 2012; Drage, 2015). The CCME has provided Canadian As and Hg guidelines for the protection of aquatic life and environmental and human health regarding Hg and As concentrations. Maximum limits have been set for Hg in sediment (0.486 ppm dw, freshwater, 0.7 ppm dw marine), soil (6.6 ppm, residential) and water (0.026 ppb freshwater, 0.016 ppb marine), as well as for As in sediment (17 ppm dw freshwater, 41.6 ppm dw marine), soil (12 ppm) and water (5 ppb freshwater, 12.5 ppb marine) (CCME, 2003). However, there are no tissue residue guidelines for the protection of life set for either Hg or As to this point.

Parsons et al. (2012) summarize results from a multi-disciplinary investigation of the dispersion, speciation and fate of metal(loid)s in terrestrial and shallow marine environments surrounding 14 of the 64 historical gold mine districts in Nova Scotia. Mercury and As analysis of 482 tailings and sediment samples from these sites showed that As ranged from 10 to 312,000 mg/kg (median = 2250 mg/kg) with 99% of samples exceeding both soil and sediment guidelines, while Hg ranged from <5 µg/kg to 350,000 µg/kg (median = 1640 µg/kg), with 20% exceeding soil guidelines, and 71% exceeding sediment guidelines (CCME, 2013; Parsons et al., 2012). Early results from this and

similar studies led to the formation of a Provincial-Federal Historic Gold Mines Advisory Committee (HGMAC) in 2005, which evaluated the ecological and human health risks associated with these gold mines in more detail, and issued warnings to help reduce human exposure to tailings at some sites (Drage, 2015; Nova Scotia Environment, 2017). The potential ecosystem and human health risks at most sites are still not well understood, and many gold mining districts remain unstudied.

The objective of this review was to critically examine and summarize existing articles, publications and reports with information on bioaccumulation of Hg and As in organisms, both aquatic and terrestrial, exposed to historical gold mining waste in Nova Scotia. This included any living organisms (excluding humans) that could be adversely affected by environmental contamination resulting from historical gold mine tailings. Until this point there has been no systematic review of bioaccumulation of Hg and/or As in gold mining or tailings-affected sites in Nova Scotia. Future research needs on this topic have also been identified.

METHODS

Documents and publications containing information about legacy gold mine contaminants and biological impacts were located using multiple sources and techniques, including online databases (Web of Science and Google Scholar) and government databases held within the Nova Scotia Department of Natural Resources, Environment and Climate Change Canada, and the Geological Survey of Canada. Reviewing the bibliographies and literature cited sections of prior reports also led to other documents. Finally, scientists and managers who work on this topic in academia, government and

private sectors were directly consulted, and many grey-literature reports and documents only available in hard-copy format were discovered in this way. These reports were digitized to PDF files and uploaded to our internal library for future reference.

At the time of writing, collected studies were produced between 1978 to 2017, with 7 between 1978-1989, 2 from the 1990s, 2 from the 2000s, and 12 from post 2010. These 23 studies included data from 16 historical gold mining districts and 14 associated reference sites (Table 1, Figure 1). We found information for several broad taxonomic groupings including: (1) freshwater, terrestrial and marine plants; (2) fungi, (3) freshwater and terrestrial invertebrates; (4) marine shellfish; (5) amphibians; (6) fish; and (7) mammals. The review below follows those same broad categories, with results discussed chronologically within each section. Values for [Hg] and [As] in organisms and relevant information (e.g., sample size, type of organism, location) were extracted from tables and text in all papers. If the authors used a reference site (e.g. non-impacted site with no gold mine tailings) to compare with the contaminated site data, we included information on those datapoints as well. We did not need to extract data from graphs in this review. Some reports included speciation data (e.g. inorganic arsenic or MeHg), but the majority reported only total As (TAs) and Hg (THg) concentrations. All Hg and As units were converted to ppm, and described in either dw or wet weight (ww).

A number of studies had limitations such as small sample sizes, or lacking specific identification of species with only common names provided. However, when possible, the scientific names were interpreted based on the context and description within the reports. Some authors had a broad approach to sampling in order to maximize the number of environmental matrices. At times, only average values or the minimum-maximum values

were reported. Longitude and latitude were not always reported, especially in older documents, so data was organized by associated district, mine and waterbody where possible.

The largest gold districts in Nova Scotia often encompass several mines and tailings areas, names after mining companies that operated in the district at a specific point in time. For example, Montague District includes both the main Montague Gold Mine site and several separately named mines and ore processing sites. There is a reporting bias within this literature review, with the largest datasets associated with the Montague District, Upper Seal Harbour (USH) District and Lower Seal Harbour (LSH) District (Table 1, Figure 3, Figure 4).

It should be noted that our literature review found no studies examining tailings-related Hg or As accumulation in lichen, reptiles, birds, or large mammals. Although there are other contaminants of concern associated with historic gold mine wastes (e.g. nickel, lead, antimony), this review is limited to Hg and As, as elements present the greatest potential risk to ecosystem and human health at gold mine sites in Nova Scotia (Drage, 2015; Parsons et al., 2012). Results of this literature review were summarized in a map depicting each study site, and the broad taxonomic groups studied at each location (Figure 1). Studies were also categorized by district to highlight the most studied locations (Table 1), and by taxonomic groups, to show the most studied species and habitats (Supplemental Information, Table 2).

REVIEW

Algae & Plants

Plants are the most commonly researched taxonomic group in Nova Scotia at legacy gold mine tailings. Nine studies document [Hg] and/or [As] in plants from tailings-impacted sites (Figure 1). These studies identified over 50 different plant species, to varying levels of taxonomic resolution. The majority of the plant-related publications pertained to terrestrial plant species (n=35 species), followed by wetland or freshwater aquatic species (n=17 species), with only one publication discussing marine flora (n=1 seaweed species; Koch et al., 2007).

The earliest report, Eaton (1978), conducted broad surveys of three gold mine districts (Montague, Mount Uniacke and Oldham) and collected ferns, grasses, mosses and rooted aquatic vegetation during the summer and early autumn of 1977. Plant samples in this study were characterized broadly (e.g., “grass”, “moss”). Mercury concentrations (ww) ranged from 0.01 to 1.05 ppm at Montague, 0.012 to 1.8 ppm at Mount Uniacke, and 0.0004 to 5.8 ppm at Oldham. Overall, aquatic vegetation had higher [Hg] than terrestrial plants, with filamentous and encrusting algae samples from Oldham demonstrated especially elevated [Hg] (up to 5.06 ppm). Although there was little evidence of a correlation between Hg in vegetation and in sediment/soil, the strongest correlation ($r^2=0.24$) was found at Oldham. No reference data were provided with this study. Terrestrial and shoreline plants (n=8 species) at Montague District were again assessed by Dale and Freedman (1982) in a detailed plant survey at various sites along the main tailings flats and Mitchell Brook (Montague District). Average [As] in plants from tailings sites ranged from 11 to 834 ppm dw (median = 101 ppm), compared to 0.5 to 6 ppm (median = 2 ppm) in reference plants from Flemming Park, NS.

Aquatic and semi-aquatic plants (n=7 species) at Oldham District were studied by Lane et al. (1988) for [Hg] and [As] concentrations in both the roots and shoots of the plants growing in or near tailing material. Average [Hg] ranged from 0.18 to 16.3ppm dw (shoots) and 0.47 to 6.11 ppm dw (roots), while average [As] ranged from 321 to 4260 ppm dw (shoots) and from 2650 to 6340ppm dw (roots) (Lane et al., 1988). Overall, both [Hg] and [As] were highest in root samples, with [As] ranging from 4.4 to 17 times higher than shoot concentrations (Lane et al., 1988). Reference data was presented for As only, ranging from 2 to 13ppm dw, with concentrations up to 300 times lower than [As] found in plants at Oldham District (Lane et al., 1988). In a subsequent report, Lane et al. (1989) conducted further analysis on these stored plant samples from Oldham (n=32 species, n=598 samples). They found that [Hg] ranged from <0.01 to 8.86 ppm dw, while [As] ranged widely from 2 to 10,000ppm dw (Lane et al., 1989). Plant roots, again, had higher [Hg] and [As] than plant shoots in all plant samples where a comparison was conducted. No reference site data was provided with this study but [As], again, far exceeded the reference data in the Lane et al. (1988) study.

Marine flora was studied only once, by Koch et al. (2007). Seaweed (*Fucus sp.*) collected from Lower Seal Harbour (LSH) District had TAs ranging from 27 to 43 ppm ww, with InAs ranging from 9.4 to 13.2 ppm. This was elevated compared to reference seaweed, with TAs ranging from 6 to 10 ppm ww, with InAs from 0.75 to 1.0 ppm. Bioaccessible As accounted for 63-81% in seaweed samples overall, and did not differ significantly between organisms at contaminated and reference sites. Authors noted that as the seaweed is attached to rocks rather than growing in contaminated sediment, the As accumulation is likely a result of uptake from the surrounding water (Koch et al., 2007).

Data from Saunders et al. (2011) also demonstrated that terrestrial plants from the Upper (USH) and Lower Seal Harbor (LSH) Districts have elevated [TAs]. Terrestrial plants at at USH District had mean TAs of 25 ppm (8.8 ppm As(III), 10 As(V)) and plants at LSH District had mean TAs of 8.8 ppm (2.4 As(III), 1.6 As(V)), while reference plants had mean TAs of 0.14 ppm (<0.04 As(III), <0.04 As(V)).

Edible plants were studied only once by Koch et al. (2013), who assessed both TAs and As bioavailability in edible berries, among other country foods (see fungi and mammal sections), growing at the LSH District. They found TAs ranged from 8 to 21 ppm ww in blueberries at the tailing site (n=4), and from 0.059 to 0.16 ppm ww in blueberries, blackberries and raspberries from uncontaminated sites (n=3). Toxic InAs accounted for a large proportion of the bioavailable fraction in these samples, and authors noted this may be a potential human health risk if berries are indeed being consumed (Koch et al., 2003).

Fungi

Edible fungi species were also included in the Koch et al. (2013) study of tailings-related contaminants in country foods. Two fungi species (*Laccaria laccata* and *Suillus luteus*) were collected from tailings sites within LSH District. Authors found that *Suillus luteus* had 0.50 ± 0.03 ppm ww TAs (n=1, duplicate extraction), while *Laccaria laccata* contained 46 ppm ww (n=1). Both species contained predominantly DMA, and *Laccaria laccata* also containing a significant amount of TMAO (Koch et al., 2013). However, as

only one sample was collected for each species, the authors concluded more data were needed before drawing conclusions on As speciation in these species, at tailings sites.

Freshwater and Terrestrial Invertebrates

Invertebrates from freshwater and terrestrial habitats were discussed in five studies, including an unpublished B.Sc. honours thesis. The majority of the studies were conducted at Montague District, with some data from USH and LSH Districts (Figure 1). Two of these evaluated [Hg] (Eaton, 1978; Robinson, 2016) while three evaluated [As] (Brooks et al., 1982; Moriarty et al., 2009; Button et al., 2012). A variety of terrestrial invertebrate species ($n=11$) and aquatic freshwater species (or life stages) ($n=5$) were described within the studies, with 14 different species or taxonomic groups identified in total.

Water striders (genus *Gerriidae*, $n=3$) collected by Eaton (1978) from Mitchell Brook within the Montague District had [Hg] ranging from 0.13 to 0.25 ppm dw. Brooks et al., 1982 also sampled aquatic invertebrates from along the tailings-impacted area of Mitchell Brook, and found that composite samples of caddisflies (genus *Trichoptera*) and mayflies (genus *Ephemeroptera*) had [As] ranging from 0.002 to 0.059 ppm dw. No reference data was included in either of these studies.

Later, freshwater invertebrates from Mitchell Brook were again sampled by Robinson, 2016, at a tailings-impacted wetland along the brook nicknamed “Old Stamp Mill” (Robinson, 2016, unpublished). Their study, although unpublished, indicated elevated [Hg] in dragonfly larvae (suborder *Zygoptera*), damselfly larvae (suborder *Anisoptera*), and aquatic spiders (genus *Dolomedes*). Organisms from Old Stamp Mill

Wetland had [Hg] up to 2.0 ppm dw, while reference invertebrates from an upstream, uncontaminated site along Mitchell Brook had mean [Hg] from 0.17 to 0.24 ppm dw.

Terrestrial invertebrates at historical tailings sites were described in two publications (Moriarty et al., 2009; Button et al., 2012). Moriarty et al. (2009) assessed [As] amongst a variety of taxonomic groups (including spiders, grasshoppers, ants, flies and earthworms) collected from the Montague, USH and LSH Districts. They found that organisms from all categories had elevated TAs compared to those at reference sites, including moths which had up to 22 ppm dw As at Montague, compared to 0.13 ppm dw at a reference site along East Brook (Moriarty et al., 2009). Earthworms (*L. castaneus* and *D. rubidus*) at the LSH District were also found to have exceptionally elevated [As] by Button et al., 2012. Concentration of TAs were up to 2200ppm dw in organisms from LSH, compared to 4.3 ppm dw at the a reference site at New Harbour (Button et al., 2012). This marked the highest [As] reported to date in earthworms.

Marine Shellfish

Five studies, to date, examine tailings-related [As] in marine shellfish tailings-impacted coastal sites, two of which also include [Hg]. The first study published on shellfish was conducted by Koch et al. (2007), who found that soft-shelled clams (*Mya arenaria*) from Seal Harbour (LSH District) had elevated TAs, ranging from 218 to 228 ppm ww, compared to reference clams (unidentified species, purchased from grocery store) with TAs ranged from 7.0 to 7.9 ppm ww. Blue mussels (*Mytilus edulis*) were later tested along a gradient of tailings contamination in Seal Harbour by Whaley-Martin et al., 2012. Results showed that composite mussel samples (n=10-15) had average TAs of 60-

109 ppm dw, with InAs of 15-33 ppm ww, while reference organisms from Coddles Harbour and a local grocery store, had TAs of 16-34 ppm dw and InAs of 0.2-7.92 ppm dw. Authors suggested that this concentration of IAs may present a risk to any higher trophic organisms (including humans) consuming the mussels (Whaley-Martin et al., 2012).

Periwinkles (*Littorina littorea*) from Seal Harbour also had exceptionally elevated TAs (Whaley-Martin et al. 2013). Average TAs concentrations in organisms from LSH ranged from 400 to 840 ppm dw, with [InAs] from 290-588 ppm dw, while reference periwinkles from Coddles Harbour had 56 ppm ww TAs, with 6.6 ppm InAs. These concentrations of As were attributed, in part, to the feeding mechanism of the periwinkles, which scrape seaweed off rocks and other substrate, and therefore are in close contact with tailings-contaminated sediment (Whaley-Martin et al., 2013). The [InAs] accumulated in these periwinkles represents, to date, the highest concentrations reported in any marine organisms. Again they were noted to be a potential risk to marine consumers (e.g. crab and bird species) and to humans harvesting edible periwinkles.

Walker & Grant (2015) also analyzed blue mussels, along with American lobsters (*Homarus americanus*) from Isaacs Harbour (LSH District). Historically, tailings were discharged directly into deeper waters in Isaacs Harbor, with minimal impact on the intertidal zone (Parsons et al., 2008). In addition, Isaacs Harbour does not receive contaminated freshwater runoff from mine tailings, which is a major source of exposure for shellfish in Seal Harbour (Milligan and Law, 2013). Accordingly, the mussels from these sites demonstrated lower [As] than those tested by Whaley-Martin et al (2012, 2013). Mussels had TAs of 1.3-2.0 ppm ww, and [Hg] of 0.02-0.05 ppm ww, while

lobster hepatopancreas tissue had TAs of 5.0-10.0 ppm ww and THg of 0.06-0.12 ppm ww. These results demonstrated significantly elevated [Hg], but not [As], found in organisms from the contaminated Isaacs Harbour site, compared to the reference Country Harbour site. Authors noted that while no mussels exceeded CFIA guidelines, a number of lobster tissue samples exceeded CFIA Hg guidelines for fish and fish products (CFIA, 2011) at both reference and contaminated sites.

Despite much study of coastal locations near USH and LSH gold districts, there were no publications for Hg or As in species at any other coastal tailings sites until 2017, when Doe et al. (2017), released a paper on blue mussels (*Mytilus edulis*) and soft shelled clams (*Mya arenaria*) collected from eight different historical gold districts. The highest [As] was found in organisms from Seal Harbour (up to 309 ppm ww), however, all 8 sites demonstrated elevated [As] when compared with reference organisms from New Harbour. Mollusks collected near the Goldenville, Gold River and Wine Harbour Districts also had elevated [As] compared to the reference site, demonstrating that other coastal tailings sites are also affecting nearby marine environments.

Amphibians

In their 1978 report, Eaton (1978) included [Hg] for a number of frogs ($n=16$) and toads ($n=1$) of unidentified species, living within the Oldham, Mount Uniacke and Montague Gold Districts. The amphibians had ranged from 0.1 to 0.45 ppm ww at Oldham ($n=4$), from 0.16 to 0.32 ppm ww at Mount Uniacke ($n=7$), and from 0.03 to 0.06 ppm ww at Montague ($n=6$), however, no reference data were provided (Eaton, 1978). Only one other publication reported on amphibians. Moriarty et al. (2013) reported on

[As] in the leg tissue of green frogs (*Rana clamitans*, n=11) and an eastern American toad (*Bufo americanus*, n=1) collected from the USH District. Amphibians at USH had elevated [TAs], ranging from 1.6 to 4.4 ppm ww (2.7 ± 1.2 ppm ww average), while reference animals had average TAs of 0.23 ± 1.2 ppm ww TAs (Moriarty et al., 2013).

Fish

The effects of historical tailings on fish species have been described in six publications, mentioning 12 predominantly freshwater species (Brooks et al., 1982; Dale and Freedman, 1982; Eaton, 1978; Eaton and Clair, 1985; Tetford, 1999; LeBlanc & Halfyard, 2010).

Eaton (1978) included a number of eel (n=14), bass (n=1), perch (n=5) and trout (n=5), collected from Montague and Oldham districts, with [Hg] of 0.12-0.75 ppm ww (Eaton, 1978). No reference data was included, but this initial study demonstrated a number of fish from both sites that surpassed the current Hg CFIA safety guideline for edible fish tissue of 0.5 ppm ww (CFIA, 2011).

An exposure experiment was later conducted by Dale and Freedman (1982), by placing 10 banded killifish (*Fundulus diaphanous*) originally caught from a non-contaminated site (Fleming Park, NS) in contained cages for four weeks in the tailings affected Mitchell Brook (Montague District). The study site was located in an area previously determined to have the highest water [As] in that stream (Brooks et al., 1982). Results showed significant As accumulation in fish caged in Mitchell Brook, (0.639 ppm ww) compared to fish caged at a reference site (0.446 ppm ww), by the end of the experiment. Two native Banded Killifish were also accidentally caught in the Mitchell

Brook cages during the experiment, and were found to have [As] of 4.02 and 4.77 ppm ww; surpassing the current TAs CFIA guidelines of 3.5 ppm ww for edible fish tissue (CFIA, 2018).

Eaton and Clair (1985) also found elevated Hg in fish caught from four tailings-affected lakes within the Waverley District. They collected brook trout (n=5), white perch n=12), small mouth bass (n=3), and white suckers (n=7), and found that Hg ranged from 0.11 to 2.00 ppm ww. Although the study lacked reference data, 15 of 27 fish samples (from a variety of species) exceeded the current recommended CFIA consumption safety guideline of 0.50 ppm ww Hg (CFIA, 2018).

White perch (*Morone Americana*) were studied by Tetford (1999), in an unpublished honors thesis, examined [Hg] in fish from Long Lake; a lake within Caribou District which is partially filled with gold mine tailings. Average [Hg] in fish from Long Lake (n=9) was 0.62 ppm, roughly 3 times that of fish from the uncontaminated Angevine Lake (n=12) which had average Hg of 0.23 ppm dw. Fish from Long Lake also had shorter average fork lengths (138.78 mm vs. 147.58 mm reference), and lower average weights (33.67 g vs. 50.33 g reference), with Long Lake fish weighing up to 40% less than fish of equal length from the reference site (Tetford, 1999). The author noted that both sites were small, land locked, oligotrophic lakes of the same region, and therefore hypothesized that differences in [Hg], size and mean fork length were potentially attributed to the influence of the tailings material (Tetford, 1999, unpublished).

Finally, in the most extensive and recent fish study in Nova Scotia, LeBlanc and Halfyard (2010) provide an in-depth analysis of contaminants in 11 fish species from 13 different tailings-contaminated lakes, compared to fish from six reference lakes (n=300

fish total). Fish from tailings-affected lakes had average [Hg] of 0.62 ppm ww (11 species, n=172), which was slightly higher, though not statistically significant, from the average [Hg] in fish from non-contaminated lakes of 0.57 ppm ww (7 species, n=45) (LeBlanc and Halfyard, 2010). Fish [Hg] was positively correlated with diet, and in some species, with length and weight. There was also some evidence that fish from tailings-contaminated lakes had higher [TAs], after a comparison of lakes using an analysis of covariance (ANCOVA), however most fish did not exceed the CFIA TAs guideline of 3.5 ppm ww (LeBlanc and Halfyard, 2010).

Small Mammals

Four mammal species (from five publications) have been studied for bioaccumulation of tailings-specific Hg and/or As in Nova Scotia. Mammals include; meadow voles (*Microtus pennsylvanicus*), shrews (*Sorex cinereus*), hares, and mice of unspecified species (Eaton, 1978; Saunders et al., 2010, 2011; Koch et al., 2013). A number of small rodents (mice of unspecified species) were first included in the the Eaton (1978) survey. Animals from Oldham, Mount Uniacke and Montague districts had [Hg] of 0.01-0.064 ppm ww (n=4), with the highest concentration found in a mouse at Mount Uniacke (Eaton, 1978).

Small rodents from Montague District were again assessed by Saunders et al. (2010), who conducted a study on meadow voles (*Microtus pennsylvanicus*) living at or near tailings flats. Vole [As] tissues ranged from 2.1 to 6.2 ppm ww (n=10), compared to 0.25 to 0.53 ppm ww (n=10) at a nearby reference site off Montague Road (Saunders et al., 2010). In 2011, Saunders et al. (2011) published similar results in voles from USH

and LSH districts, where the animals had [TAs] of 0.52-2.5 ppm ww, compared to 0.19-0.48 ppm ww at a reference site (Saunders et al., 2011). The animals were known to feed mainly on terrestrial invertebrates, which were previously shown to be highly contaminated at both Montague, USH, and LSH gold districts (Moriarty 2009; Button et al., 2012). Accordingly, prey items and stomach contents of the voles were analyzed, and elevated [As] were found; 7.6 ppm ww in animals from Montague, and 4.2 ppm ww in animals from the Seal Harbour area, compared to 0.44 and 0.48 ppm ww in animals from reference sites, respectively (Saunders et al., 2010, 2011). Similarly, Moriarty et al. (2011) showed that shrews (*S. cinereus*) from USH also had elevated mean As when compared to a control site (0.28 ppm ww vs. 0.1 ppm ww).

Finally, hunted game species living near tailings sites were assessed in only one study. Koch et al. (2013) in their survey of foraged and hunted ‘country foods’, found that TAs in a hare caught near tailings at Seal Harbour (LSH District) had 0.021 ppm ww (n=1), compared with animals from reference sites, which had TAs of 0.44 and 0.007 ppm ww (n=2). Bioaccessible As in hare tissue was predominately InAs, although some samples with lower TAs had InAs below detection limits. Authors noted that the tissue was analyzed uncooked, but that cooking would likely occur prior to human consumption, which may change As speciation (Koch et al., 2013).

Non-Tailings Related Studies

Although this is the extent of studies specifically linked to tailings-sourced Hg and As, there have been a number of other studies demonstrating elevated [Hg] in mammals and bird species within Nova Scotia.

In a 2015 study by Little et al., elevated [Hg] were found in fur samples of little brown bat collected from Nova Scotia when compared with Canadian background levels (Government of Canada, 2016). The study included fur samples from animals at many locations across Atlantic Canada, but only two bats were collected specifically within historical tailings districts. Fur samples from these two animals had [Hg] of 15.7ppm ww in a specimen caught within Rawdon District, and 5.68ppm ww in one from the Chezzetcook District. In stable isotope analysis, bats from Atlantic Canada were found to have mean $\delta^{13}\text{C}$ consistent with freshwater invertebrates in the area. However, given the small sample size, we are not able to assess whether the elevated Hg was connected with historical tailings deposits. In general, the bats sampled from Nova Scotia had the highest [Hg] in all of Atlantic Canada (Little et al., 2015). Background [Hg] in little brown bats from Ontario and Quebec had [Hg] of 5.2 and 2.4 ppm ww, respectively (Government of Canada, 2016). The effects of tailings-related contaminants may be even more important to understand now, after the rapid decline of bat populations following the outbreak of white-nose-syndrome and their inclusion on the endangered species list in Nova Scotia in 2013 (DNR, 2013).

Spencer et al. (2011) examined Hg accumulation in the brains of river otters (*Lontra Canadensis*) in Nova Scotia. Brain tissues from otters (n=66) showed [Hg] ranging from 0.3 to 18 ppm ww. Although this was not directly linked to tailings-related sources, some of the animals were collected from areas with a high density of abandoned mines (Spencer et al., 2011). On a national scale, Nova Scotia is one of the only provinces where [Hg] in the livers of mink and otter had been shown to surpass the threshold for neurochemical effects (15 to 51ppm dw) (Government of Canada, 2016).

No studies were found for tailings-related contaminants in birds, however, a number of studies have demonstrated elevated Hg in birds across Nova Scotia when compared with national background concentrations. Edmonds et al. (2010) conducted a study on [Hg] in blood and feather samples of the rusty blackbird (*Euphagus carolinus nigrans*), an endangered species within Nova Scotia (Environment & Climate Change Canada, 2015), and a wetland obligate bird, who's feeding and breeding range spans the whole of Nova Scotia. Bird collected from Acadian forests demonstrated mean blood [Hg] of 1.06ppm ww, and a geometric mean of 8.26 ppm ww in feather samples (n=59) (Edmonds et al., 2010). The study included specimens from New Brunswick, New Hampshire, Maine and Vermont, but the highest overall blood [Hg] of 3.42 ppm ww, was found in a bird from Nova Scotia (Edmonds et al., 2010). The [Hg] in this animal far surpassed minimum levels know to result in adverse health effects in birds (Evers et al. 2008). In addition, MeHg accounted for approximately 98% of the THg in blood samples, and 97% in feather samples (Edmonds et al., 2010). Although the elevated mercury levels found in this species were not directly linked to historic tailings sources, historic gold mine tailings were listed as a possible cause of the elevated THg and MeHg levels found in the birds (Edmonds et al., 2010).

Finally, a number of studies have shown elevated [Hg] and [MeHg] has been found in invertebrates, fish and loons within Kejimikujik National Park, NS (Burgess et al., 2007; Buckland-Nicks et al., 2013). Burgess et al. (2007) demonstrated that as fish and loon blood [Hg] increased, productivity decreased in the bird. Although elevated [Hg] in this area has not been directly linked to historical tailings deposits, there are a number of gold mine Districts surrounding the park area.

DISCUSSION

In total, 23 studies completed since 1978 were reviewed; examining [Hg] and [As] in living organisms collected at, or near, historical gold mine tailings sites in Nova Scotia. Many of these sites have elevated [Hg] and [As] in water, sediment and soil, often in concentrations surpassing safety guidelines for the protection of aquatic life and environmental health (Eaton, 1978; Brooks et al., 1982; Wong et al., 1999; Parsons et al., 2012). Of the studies reviewed, 19 gold mining districts were mentioned in total. Lower Seal Harbour, Montague and Upper Seal Harbour districts was the most-studied locations (n=11) and studies on plants, invertebrates, marine shellfish, amphibians and fish were all conducted at this site. Seal Harbour also remains the only site to be closed to food harvesting, after early findings on mollusk contamination led to a ban on shellfish harvesting in 2005 (Environment Canada, 2005). Montague was the second most studied site (n = 7), followed by the Oldham District (n=3).

Reference data was included in 15 studies, and in all cases, organisms from tailings-impacted sites exceeded [Hg] and/or [As] found in reference organisms (Dale and Freedman, 1982; Lane et al., 1988; Tetford, 1999; Koch et al., 2007; Koch et al., 2013; Moriarty et al., 2009, 2011, 2013; LeBlanc and Halfyard., 2010; Sauders et al., 2010, 2011; Button et al., 2012; Whaley-Martin et al., 2012, 2013; Robinson, 2016; Doe et al., 2017). Although there is currently no maximum acceptable limit for Hg or As for the protection of wildlife consumers, there are numerous examples of species collected near tailings sites with [Hg] known to result in adverse health effects (Government of Canada, 2016; Edmonds et al., 2010; Evers et al. 2008; Spencer et al., 2011). The data summarized in these publications indicate that there is considerable evidence to suggest

Hg and As are also bioaccumulating in plants, invertebrates, amphibians, fish and mammals living near these sites. However, more research is needed to fully understand the effects of gold mine tailings on both species and ecosystem health.

Plants are the most studied taxonomic group at historical tailings sites, with nine different studies on tailings-related contaminant bioaccumulation, and data collect on plants across nine different historical districts (Figure 1). There is strong evidence that plants growing in tailings, soil or sediment at gold mine sites have elevated [As]. Maximum [TAs] in plants from the Oldham (6340 ppm dw), Montague (834 ppm dw) and Upper and Lower Seal Harbour (34 ppm ww) districts far exceed maximum [TAs] found in plants from associated reference sites (13 ppm dw, 6 ppm dw, 0.93 ppm ww, respectively). Studies on Hg are sparser, and baseline [Hg] for plant tissue in Nova Scotia is lacking and needed to contextualize plant [Hg] findings from the Montague, Mount Uniacke and Oldham districts. There is evidence that coastal tailings sites are influencing marine flora, and resulting in contaminant accumulation (Doe et al., 2017; Whaley-Martin et al., 2012, 2013).

Terrestrial and aquatic invertebrates from tailing sites have also been shown to have elevated [Hg] and [As]. However, studies are currently limited to three districts; Montague, USH and LSH. Aquatic invertebrates from the “Old Stamp Mill” wetland at Montague demonstrate up to 8 times higher [Hg] than at associated reference organisms (Robinson, 2016, unpublished). Terrestrial invertebrates at tailings sites had [As] over 160 times (Montague) and 300 times (USH and LSH) higher than reference organisms (Moriarty et al., 2009; Button et al., 2012). Evidence of contaminant transfer to small mammals feeding on terrestrial invertebrates was demonstrated at these sites as well, as

discussed in the mammal's section (Moriarty et al., 2011, 2013; Saunders et al., 2010, 2011). Invertebrates are common ecological receptors of contaminants as they live in direct contact with soil, water and/or sediment and have an especially high surface area-to-mass ratio. Furthermore, emergent (i.e. hatching) insects with aquatic life stages have been shown to transfer contaminants such as Hg and As to terrestrial ecosystems (Tweedy et al., 2013; Mogren et al., 2013). In this way, they can act as biovectors of contaminants to higher trophic levels and to surrounding ecosystems. (Eaton & Clair, 1985; Koch et al., 2007; LeBlanc and Halfyard., 2010; Doe et al., 2017). More research on the role of invertebrates in the transfer of tailings-related contaminants to higher predators is needed.

Marine invertebrates have also been shown to be affected by coastal tailings. Many shellfish (e.g., molluscs, crustaceans, and echinoderms) are commonly harvested for consumption along the coast of Nova Scotia by residents (Fisheries & Oceans Canada, 2018). Unfortunately, a number of popular shellfish harvesting sites along the eastern coast of the province are also known areas of historical gold mine tailing contamination. These sites include: Seal Harbour (LSH District), Isaacs Harbour (Isaacs Harbour District), Wine Harbour (Wine Harbour District), Harrigans Cove (Harrigans Cove District) and Gegogan Harbour (Goldenville District) among others (Parsons et al., 2012; Doe et al., 2017). The Canadian Food Inspection Agency (CFIA) currently has no set guidelines for Hg or As limits in shellfish, but does provide guidelines for fish tissue, limiting concentrations to 0.5 ppm ww Hg, and 3.5 ppm ww As (CFIA, 2011, 2018). Maximum [IAs] limits for shellfish have also been set in New Zealand (2 ppm ww), Hong Kong (10 ppm ww), and Australia (1 ppm ww) (Edmonds & Francesconi, 1993). Seal

Harbour clams, mussels and periwinkles had [InAs] far surpassing these levels (Doe et al., 2017; Koch et al., 2007; Whaley-Martin et al., 2012, 2013; Walker and Grant, 2015).

Prior to 2005, shellfish from Seal Harbour were commonly harvested for food by residents in the area, but preliminary data from Doe et al. (2017), later presented in this section, led to a ban on harvesting bivalve mollusks at Seal Harbour in 2005 (Contaminated Fisheries Prohibition Order No. STN-20050007; Environment Canada, 2005). At present, this remains the only location in Nova Scotia where harvesting of wild foods has been restricted due to contamination from gold mine waste (Environment Canada, 2007; Parsons et al., 2012). Additional studies on marine mollusks are needed to better understand contamination at other less-studied coastal tailings sites and to assess the risk these mollusks may present to higher-order consumers (Whaley-Martin et al., 2012, 2013; Walker & Grant, 2015).

Amphibians are often used as bioindicators of environmental conditions due to their physiology (thin skin, cutaneous respiration) and lifecycle, which includes stages in both freshwater and terrestrial environments (Moriarty et al., 2013). Although studies on amphibians are limited, with only two reports, data presented by Moriarty et al. (2013) shows elevated [As] in amphibians from USH District compared to reference organisms.

Fish are economically important for Nova Scotia, and consumed by both humans and wildlife. Sportfishing in Nova Scotia generates annual revenues of over \$58 million, with nearly 67,000 licensed anglers, the majority of whom are permanent residents of the province (Nova Scotia Depart. of Fisheries & Aquaculture, 2018). Overall, fish are the second most studied category of species, however, fish can be challenging bioindicators for understanding overall site-specific contamination, as many species in Nova Scotia are

highly mobile and migratory, possibly traveling outside the spatial bounds of the contaminated sites to feed. As a result of this behavior, fish species may also act as important biovectors of contaminants beyond the tailings sites themselves.

The reports gathered on fish species here show that there have been numerous cases of [Hg] in fish surpassing the CCME and CFIA guidelines at the Montague District (Eaton, 1978; Brooks et al., 1982; Dale & Freedman, 1982), Oldham District (Eaton, 1978), Waverley District (Eaton and Clair, 1985), and Caribou District (Tetford, 1999). However, LeBlanc and Halfyard (2010) also demonstrated a lack of overall elevated average [Hg] in fish from tailings-impacted lakes across Nova Scotia, when compared with reference sites. More research is needed on the role fish may play in the transfer of Hg and As from tailings-impacted sites to wildlife and human consumers.

California, similar to Nova Scotia, has an extensive history of historical gold mining dating back to the 1800s. As a result of publications demonstrating elevated Hg in both fish and invertebrates associated with historical and active gold mine sites (Klasing & Brodberg, 2003, 2004; Slotton et al. 1995), California issued fish consumption advisories for over 20 waterbodies affected by gold mining activities (California Depart. of Conservation, 2002). The state has also produced public documents warning the public about waterbodies that are known as tailings-related mercury “hot spots” (OEHHA, 2018). In 1997, the California Department of Conservation created the federally funded Abandoned Mine Lands Program (AMLPL) to address the health, safety and contamination concerns associated with historical mines (California Depart. of Conservation, 2002).

Nova Scotia does provide human fish consumption guidelines with the goal of limiting mercury exposure in the yearly Sportfishing Anglers Handbook (Nova Scotia

Depart. of Fisheries & Aquaculture, 2018). However, while site specific warnings are in place for lakes with elevated PCBs, there are not yet site-specific warnings for lakes or waterbodies connected to historical gold mine tailing deposits, which may be mercury and/or arsenic “hotspots” (Nova Scotia Depart. of Fisheries & Aquaculture, 2018). This is despite the fact that [Hg] surpassing the provincial and federal safety guidelines have already been found in fish from numerous districts (see Fish section).

Finally, small mammals and rodents living near tailings sites demonstrate elevated [Hg] and [As] at Montague, USH and LSH Districts, but have not been studied to date in other locations. Only one study was found which included species commonly hunted for consumption in Nova Scotia (Koch et al., 2013), and no studies on larger mammals (i.e. deer, moose) have been conducted.

It is evident from this review that there is are still significant gaps in our understanding of ecosystem impacts from historic gold mine tailings, and the transfer of Hg and As from tailings into local food webs. A lack of data for many of the gold mining districts and species living at them, makes it difficult to assess the degree to which tailings-exposed ecosystems are being affected compared to non-contaminated watersheds and terrestrial habitats within the province. More studies are especially important in cases where there are potential human health concerns. For example, in areas where activities like wild berry picking, hunting game, shellfish harvesting, or fishing may be taking place. In order to develop successful management and remediation practices for these tailings sites, we must study species- and ecosystem-level contaminant concentrations and effects.

CHAPTER 1 FIGURES

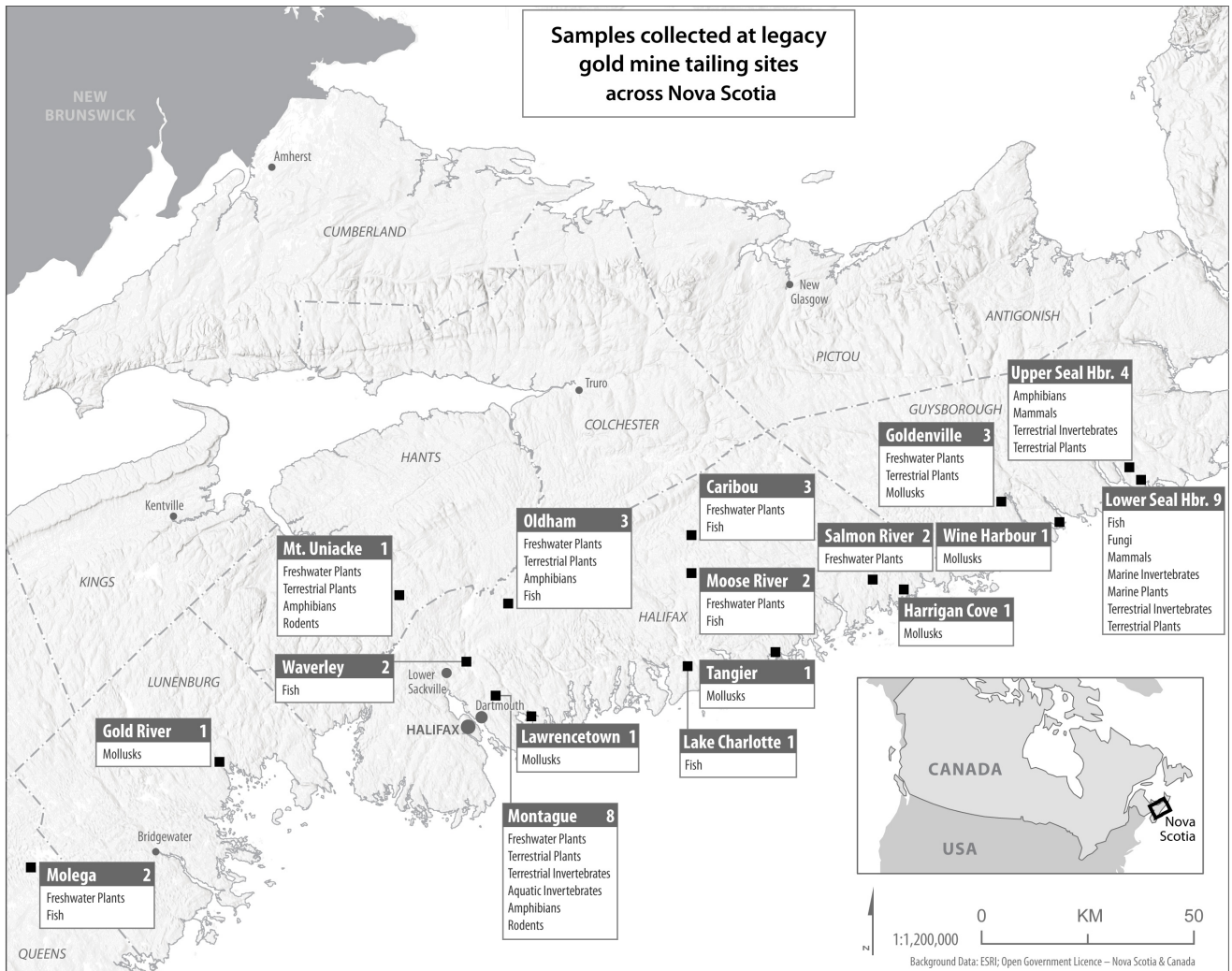


Figure 1. Summary map of historical gold mine districts, and associated broad taxonomic groups, included in literature review of tailings-related Hg and As accumulation. Map created with the assistance of Will Flanagan, Saint Mary’s University. See data breakdown in Table 1.

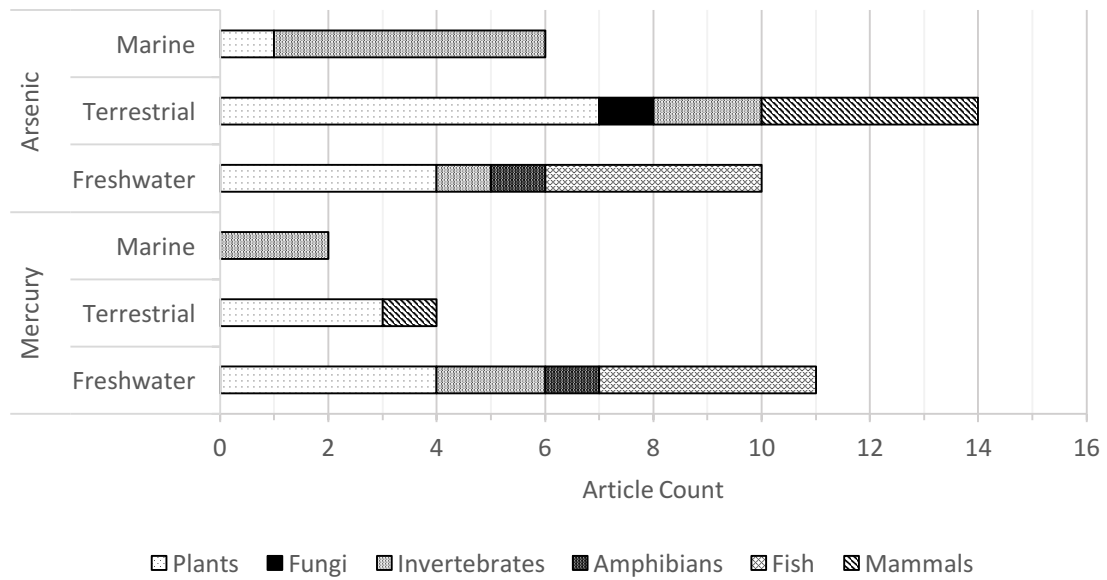


Figure 2. Count of habitats and taxonomic groups mentioned in 23 articles found describing [Hg] and/or [As] in species collected at (or in association with) historical gold mines sites in NS.

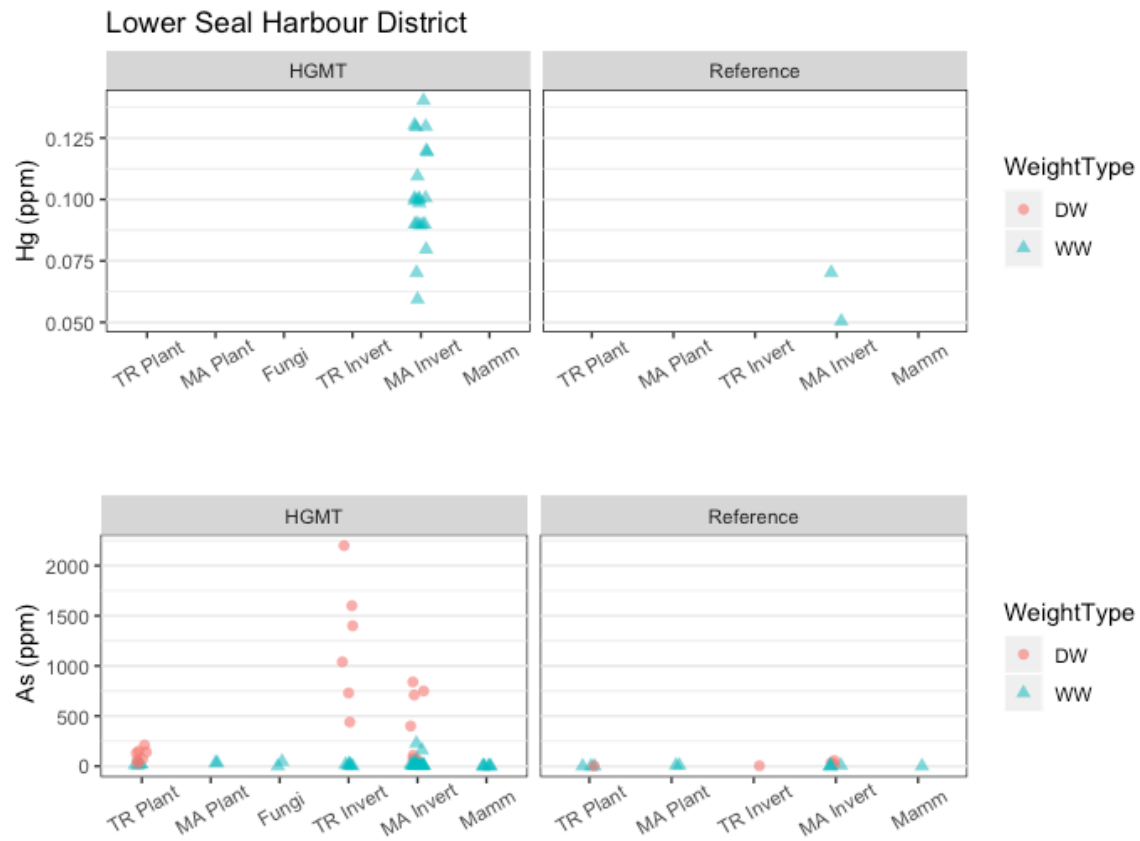


Figure 3. Summary of [Hg] and [As] data collected at the Lower Seal Harbour historical gold mining tailing site (HGMT), the most studied site within Nova Scotia. Data points are separated by wet (ww) and dry weight (dw). Sources are described in Table 1. TR – Terrestrial, MA – Marine.

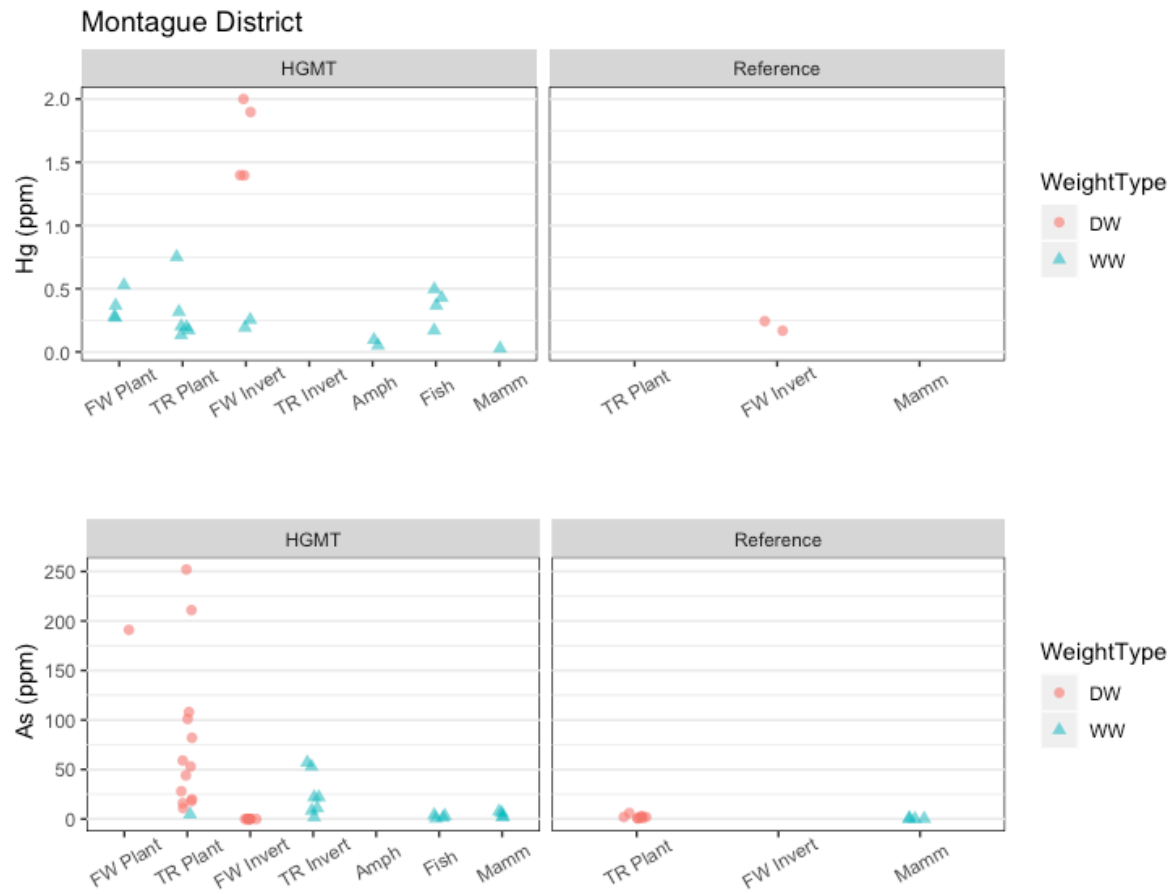


Figure 4. Summary of [Hg] and [As] data collected at Montague historical gold mining tailings site (HGMT), the second most studied site within Nova Scotia. Data points are separated by wet (ww) and dry weight (dw). Sources are described in Table 1. One outlier As concentration from Dale and Freedman (1982) removed (slender rush, 834 ppm As). TR – Terrestrial, FW – Freshwater.

CHAPTER 1 TABLES

Table 1. Timeline and summary of the 23 articles found in literature review describing [Hg] and/or [As] in ecological receptors at (or in association with) historical gold mines districts in Nova Scotia.

Article	Gold District(s)	Taxonomic Group(s)	Sample Information
Eaton (1978)	Montague, Mount Uniacke, Oldham	Aquatic Plants (n=40) Terrestrial Plants (n=53) Freshwater Invertebrates (n=3) Amphibians (n=17) Fish (n=28) Mammals (mice) (n=4)	<ul style="list-style-type: none"> Identified to common names Sample tissue not specified
Brooks and Ryan (1981)	Montague, Moose River, Caribou, Salmon River, Goldenville, Molega	Aquatic Plants (n=6) Terrestrial Plants (n=1)	<ul style="list-style-type: none"> Identified to species Sample tissue not specified
Brooks et al. (1982)	Montague	Freshwater Invertebrates (n=7, composite samples of 10) Fish (n=3, composite samples of 5)	<ul style="list-style-type: none"> Identified to common names Analyzed whole
Dale and Freedman (1982)	Montague	Terrestrial Plants (n=15, n=7 Ref, composite samples of 5) Fish (n=2)	<ul style="list-style-type: none"> Identified to species Sample tissue not specified
Eaton and Clair (1985)	Waverley	Fish (n=27)	<ul style="list-style-type: none"> Identified to species Analyzed filets
Lane and Graves (1988)	Oldham	Freshwater plants (n=12, n=2 Ref, composite samples of 5+)	<ul style="list-style-type: none"> Identified to species Analyzed roots and shoots separately
Lane et al. (1989)	Oldham	Freshwater Plants (n= 9, composite samples ranging from 5 to 74) Terrestrial Plants (n=23, composite samples ranging from 6 to 43)	<ul style="list-style-type: none"> Identified to species Sample tissue not specified
Wong et al. (1999)	Goldenville	Freshwater plants (n=unspecified), Terrestrial plants (n=unspecified)	<ul style="list-style-type: none"> Identified to species Analyzed stems and leaves

Tetford (1999), Unpublished	Caribou	Fish (n=9, n=12 Ref)	<ul style="list-style-type: none"> • No reference data provided • Identified to species • Sample tissue not specified
Koch et al. (2003)	Lower Seal Harbour	Terrestrial plants (berries) (n=4, n=2 Ref) Fungi (n=2) Mammals (hares) (n=2, n=1 Ref)	<ul style="list-style-type: none"> • Identified to common names, except mushrooms to species • Sample tissue not specified
Koch et al. (2007)	Lower Seal Harbour	Marine mollusks (n=unspecified) Marine plants (n=unspecified)	<ul style="list-style-type: none"> • Identified to species • Sample tissue not specified • No reference data provided
Moriarty et al. (2009)	Montague, Lower and Upper Seal Harbour	Terrestrial invertebrates (n=21, n=7 Ref, composite samples of unknown amount)	<ul style="list-style-type: none"> • Identified to order • Analyzed whole
Saunders et al. (2010)	Montague	Mammals (voles) (n=10, n=10 Ref)	<ul style="list-style-type: none"> • Identified to species • Analyzed by tissue type (stomach components, digestive, non-digestive, liver)
LeBlanc and Halfyard (2010)	Montague, Lower Seal Harbour, Moose River, Molega, Lake Charlotte, Waverley, Caribou	Fish (n=184, n=51 Ref)	<ul style="list-style-type: none"> • Identified to species • Sample tissue not specified
Saunders et al. (2011)	Upper Seal Harbour, Lower Seal Harbour	Terrestrial plants (n=12, n=9 Ref) Mammals (voles) (n=17, n=22 Ref)	<ul style="list-style-type: none"> • Identified to species • Analyzed by tissue type (stomach components, digestive, non-digestive, liver)
Moriarty, et al. (2011)	Upper Seal Harbour	Mammals (shrews) (n=12, n=9 Ref)	<ul style="list-style-type: none"> • Identified to species • Analyzed whole
Button, et al. (2012)	Lower Seal Harbour	Terrestrial invertebrates (n=unspecified) Leaf Litter (n=unspecified)	<ul style="list-style-type: none"> • Invertebrates identified to species • Analyzed whole
Whaley-Martin, et al. (2012)	Lower Seal Harbour	Marine Mollusks (n=4, composite samples of 10-15, n=2 Ref, composite samples of 13-15)	<ul style="list-style-type: none"> • Identified to species • Analyzed whole (shells removed)
Whaley-Martin, et al. (2013)	Lower Seal Harbour	Marine Mollusks (n=4, composite samples of 100-201, n=1 Ref, composite samples of 100-203)	<ul style="list-style-type: none"> • Identified to species • Analyzed whole (shells removed)

Moriarty et al. (2013)	Upper Seal Harbour	Amphibians (n=7, n=5 Ref)	<ul style="list-style-type: none"> • Identified to species • Analyzed legs
Walker and Grant (2015)	Lower Seal Harbour	Marine Invertebrates (n=unspecified, n=40 Ref)	<ul style="list-style-type: none"> • Identified to species • Analyzed lobster hepatopancreas, mussels whole (shell removed)
Robinson (2016) Unpublished	Montague	Terrestrial Invertebrates (n=73+, n=47 Ref)	<ul style="list-style-type: none"> • Identified to genus or suborder • Analyzed whole
Doe et al. (2017)	Lower Seal Harbour, Goldenville, Gold River, Harrigans Cove, Lawrencetown, Salmon River, Tangier	Marine Mollusks (n=20, n=5 Ref)	<ul style="list-style-type: none"> • Identified to species • Analyzed mollusk tissue

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Chapter 2. Aquatic invertebrate bioaccumulation of arsenic and mercury from legacy gold mine tailings in Nova Scotia

ABSTRACT

An estimated 3,000,000 tonnes of gold mine tailing waste were produced in Nova Scotia between the 1860s and the 1940s. Today these historical tailings deposits often overlap with aquatic and wetland habitat, and typically have elevated mercury (Hg) and arsenic (As) concentrations. We assess Hg and As accumulation in aquatic invertebrates from five tailings-affected wetlands and two reference sites. Invertebrates are often in direct contact with soil, water and sediment, and therefore can be at high risk for bioaccumulation of environmental contaminants. We found that aquatic invertebrates from all five tailings sites had significantly elevated [Hg]; average concentrations ranged from 0.76 ± 0.64 ppm dw to 4.20 ± 2.33 ppm Hg at tailings sites, while those from the reference sites only had 0.21 ± 0.21 ppm to 0.35 ± 0.25 ppm Hg. Average [As] in invertebrates was also elevated at two tailings sites. In addition, sediment [Hg] and [As] at all tailings sites surpassed probable effect levels (PEL) set by the Canadian Council of Ministers of the Environment (CCME). Water samples from tailings sites had [As] above CCME guidelines at all tailings sites, with the highest concentrations up to 1200ppb total As, and 410ppb dissolved As. This persistent environmental contamination represents a severe risk, to not only aquatic invertebrates, but to other species living at or near historical gold mine tailing deposits.

INTRODUCTION

Gold mining had a significant influence on the development and growth of the province of Nova Scotia since the mid-1800s. Following the original discovery of gold near Mooseland in 1858 (Malcolm, 1929), the industry quickly expanded, undergoing three major gold rushes until the 1940s. This resulted in a total of 64 gold mining districts (containing hundreds of individual mines) scattered across the mainland of the province. An estimated 1.2 million troy ounces of gold were produced over the lifespan of the industry (Bates, 1987). However, since the mid 1940s, most companies owning these mining districts have disbanded, and the mines themselves have been abandoned (Parsons et al., 2012). A detailed history of the industry can be found in Bates (1987), Parsons et al. (2012) and the Art Gallery of Nova Scotia (2013).

From the 1850s to the 1940s, gold ore in Nova Scotia was crushed using a stamp mill or ballmill. The rock was first crushed to a silt- to sand-size particles, and then the gold extracted with the use of mercury (Hg) amalgamation techniques (Blakeman, 1978). The use of Hg often led to contamination of the nearby environment as the Hg recovery process was inefficient during this era, and an estimated 10 to 25% of the Hg was lost to the atmosphere, or to the tailings, which were deposited nearby (Eaton, 1978; Parsons & Percival, 2005). In addition, arsenopyrite (FeAsS) occur naturally within auriferous veins in Nova Scotia (Kontak & Jackson, 1999; Parsons et al., 2012). Therefore, when the FeAsS-rich tailings were deposited on the surface, oxidation often led to the release of arsenic [As] into the surrounding aquatic and terrestrial environments (Lengke et al., 2009; Parsons et al., 2012).

Approximately 3,000,000 tonnes of gold mine tailing waste material is estimated to remain throughout the province today, much of it containing elevated [Hg] and [As] (Parsons et al., 2012). Because tailings were generally deposited near the mine sites (which required a freshwater source), tailings deposits often overlap with wetland and aquatic environments. Wetland conditions can lead to the formation of organic Hg compounds such as methylmercury (MeHg) (O'Driscoll et al., 2005). Methylmercury is highly toxic, and is both bioavailable and slow to be eliminated, creating a risk for biomagnification (O'Driscoll et al., 2005). Rapid biomagnification of MeHg, leading to harmful effects in top predators such as fish and fish-eating species, has been observed in numerous freshwater and marine food webs (Lavoie et al., 2013).

Arsenic has a highly complex chemistry with more than 50 different species identified, which range from extremely toxic to completely non-toxic (Rahman & Hasegawa, 2012). Toxicity depends greatly on bioavailability, concentration, speciation, and detoxification mechanisms of exposed organisms (Cullen & Reimer, 1989; Francesconi & Kuehnelt, 2004). In general, inorganic arsenic (IAs) forms are more toxic than organic forms, as they are proven carcinogens (Mass et al., 2001; Ng, 2005). In some studies, IAs has been found to enter freshwater systems following erosion of tailing material (Campbell & Nordstrom, 2014).

Since 1978, there have been a number of studies of soil, sediment and water contamination at, near, or downstream of historical gold mine tailing sites in Nova Scotia (Brooks et al., 1982; Eaton, 1978; Parsons et al., 2012; Wong et al., 1999). From 2003 to 2012, Parsons et al. conducted a study of soil, water and sediment contamination at historical gold mine districts. Hg and As consistently exceed probable effect levels

(PELs) for the protection of aquatic life and environmental health in tailings impacted water and sediment (CCME, 2003; 2014). Few studies have been done to assess the transfer of tailings-related As and Hg from tailing material to aquatic and terrestrial wildlife. In addition, a large number of the districts remain completely unstudied.

Aquatic invertebrates live in direct contact with soil, water and sediment, and therefore, can be at risk for bioaccumulation of contaminants present in the environment. Indeed, aquatic invertebrates living near contaminated sites have often been shown to accumulate high concentrations themselves, and to transfer these contaminants to higher predators (Blais et al., 2007; Mogren et al., 2013; Torres & Johnson, 2001, Jackson et al., 2011, Speir et al., 2014). Some previous research suggests this may also be occurring at historical gold mine tailings sites in Nova Scotia (Brooks et al., 1982; Eaton, 1978; Robinson, 2016), however, within these past studies, sample sizes were often small and data were only collected at the Montague, Upper Seal Harbour and Lower Seal Harbour gold mining districts (Chapter 1). This study examines [Hg] and [As] in sediment, water and freshwater invertebrates at five tailings-impacted wetlands, and by doing so expands our understanding of the ecological risk historical tailings deposits pose to surrounding food webs.

METHODS

Data collection took place throughout the growing seasons (May-August) of 2016 and 2017. Sampling sites were selected from gold districts ranked among the 25 historically most productive sites in Nova Scotia. Five contaminated wetland/aquatic sites and two reference sites (Table 1) were selected based on wetland habitat, accessibility,

and on historical mining production data for the contaminated sites (Parsons et al., 2012). The two reference sites (Mitchell Brook and New Dam Flowage River) were located upstream of the Montague and Moose River tailings sites, respectively. In this paper, we use abbreviations to describe each site, which can be found in Table 1.

Four adjoining 4.57 x 4.57 m quadrants were defined alongside the shoreline of the wetland sampling sites (labelled 1-4). This was done in order to assess variation in contamination on both a smaller (quadrant) and larger (site) scale. At the OS site, insufficient aquatic invertebrates were found within the quadrant, therefore larger quadrants of 15 x 15 m were used in order to collect enough sample mass for analysis (Figure 1). At reference locations, greater quantities of invertebrates were found, therefore, only two 4.57 x 4.57 m quadrants were used to conserve sampling time.

Bulk sediment samples were collected to 15 cm depth were collected from all quadrants using a short PVC pipe sediment corer, and divided into three segments: 0-5 cm, 5-10 cm, and 10-15 cm depths. Samples were kept on ice during transport back to the lab, where they were frozen immediately. The samples were eventually thawed and dried at approximately 55 °C to avoid loss of Hg (Heiri, et al., 1999), lightly disseminated using a mortar and pestle, and sifted using a 2 mm filter to remove coarse organic matter.

Water chemistry data were collected at each quadrant using a calibrated YSI multi-parameter water sonde (Parameter Plus model). Parameters recorded included pH, dissolved oxygen (% DO), water temperature (degrees Celsius), salinity, total dissolved solids (TDS) and specific conductance (SPC). Water samples were also collected in October 2017 using the “clean-hand, dirty hands” technique in order to avoid external

contamination. Sub-samples for total mercury were preserved using ultrapure nitric acid in Teflon bottles, immediately following collection.

Aquatic invertebrates were collected from each quadrant using a kicknet during June to July of 2016 and 2017. Common taxa of invertebrates collected included snails (phylum *Mollusca*, class *Gastropoda*), mussels and clams (phylum *Mollusca*, class *Bivalvia*), leeches (phylum *Annelida*, class *Hirudinae*), water mites and spiders (phylum *Arachnida*), and a wide variety of taxa within the phylum Arthropoda such as mayflies (order *Ephemeroptera*), dragonflies and damselflies (order *Odonata*), and beetles (order *Coleoptera*). Invertebrates were transported back to the lab on ice, left alive for a 24-hour gut purge, and washed with squirt bottle with RO water (US EPA, 2000; MOECC, 2006). They were also visually inspected for sediment particulates under a microscope, before being frozen at -20°C.

Prior to analysis, all samples were thawed, washed with RO water, and visually inspected under a dissecting scope for remaining sediment particulates. Samples were identified to genus when possible, and weighed to collect wet weight (ww) data. They were then dried overnight at a low temperature (50-55 °C, Schmidt et al., 2013), and weighed again to collect dry weight (dw) data, in order to calculate moisture content. Due to the large quantity of samples, invertebrates from the same genus and quadrants were pooled as composite samples and homogenized to a fine powder using a Retsch mixing mill. The only exception was samples with very low mass, which were left whole to avoid loss of sample material.

Total [Hg] for all invertebrate samples was measured using a Milestone Direct Mercury Analyzer (DMA) 80.3 in a clean-room laboratory at Saint Mary's University.

Trace-element protocols were followed to ensure no cross contamination of samples occurred. Samples were run in cleaned quartz sample boats. Each analysis run was preceded with multiple blanks, a series of aqueous mercury standards (0, 5, 10, 15, and 20 ppm), along with 2-3 certified reference materials (CRM), in order to ensure calibration accuracy. CRMs used for invertebrate runs included NIST2976, TORT2, TORT3, DORM4 and DOLT5. Each sample was placed in an acid-washed, quartz sample boat, weighed and recorded on the DMA80 computer software. To prevent contamination carry-over, two blanks were run between invertebrate samples from differing quadrants, and a full set of blanks were run between site locations. All [Hg] data is presented as ppm of dry weight (dw).

A subsection of invertebrate samples (n=58) from tailings and reference sites were sent for As analysis. Samples consisted of four genera; *Sympetrum* and *Aeshna* (dragonfly larvae), *Hyelella* (amphipod crustaceans) and *Dolomedes* (aquatic-feeding spiders). During As analyses, a number of samples (n=24) were reported to have [As] below method detection limits (MDL) as a result of having low mass, with MDL's varying widely from 8.8 to 660ppm. All [As] data is presented as ppm of dry weight.

To avoid equipment contamination in our laboratory by extremely elevated metal(loid) concentrations, all sediment samples were sent to Bureau Veritas laboratory for Hg, As, and additional element analyses via ICP-MS (method code AQ250; 1:1:1, HCl: HNO₃: H₂O digestion ultratrace ICP-MS). A number of sample duplicates were run to ensure consistency, along with blanks, and standard materials (DS10 and OREAS4EA). Some samples (n=12) surpassed both the Hg and As maximum detection limits, and were then re-analyzed using ICP-ES (method code AQ370; Aqua Regia – ICP-

ES). Total carbon (TC) and total organic carbon (TOC) were analyzed using a Leco CHNS Tru-Spec analyzer at the Geological Survey of Canada (Dartmouth, Nova Scotia). Loss-on-ignition (LOI) values were calculated using gravimetric methods and a muffle furnace (Heiri et al., 1999).

Water samples were sent to the Analytical Services Unit, Queen's University where filtered and unfiltered samples were analyzed using ICP-MS. Blanks and controls were included with runs, with scandium, indium and bismuth used as internal standards. Water samples were also sent to the Environmental Isotope Laboratory, Waterloo University where they were analyzed for dissolved organic carbon (DOC) in both filtered and unfiltered samples via isotope analysis. The results presented are an average of three replicates for each sample. Water was also analyzed for THg with Milestone DMA 80.3.

Statistical analysis was conducted using RStudio. Log_{10} transformations of Hg and As data removed heterogeneity of variance, prior to analysis, however figures demonstrate actual values. Mercury data was found to be non-parametric, therefore the Kruskal-Wallis Test was used, along with Spearman's rank-order correlation. Arsenic data was left-censored, due to variation in method detection limits, and non-parametric. Therefore, Kaplan-Meier analysis was used.

RESULTS

Mercury

Sediment [Hg] was significantly higher at tailings-impacted wetlands than at reference wetlands for all tailings sites except MR (Table 2, Figure 1). The OS site, in particular, demonstrated exceptionally high [Hg] at each of the sampled depths; $92.50 \pm$

55.0ppm (0-5 cm depth), 66.7 ± 20.4 ppm (5-10 cm depth) and 32.2 ± 26.6 ppm (10-15 cm depth). The highest overall sediment [Hg] value recorded was 140 ppm, (OS, quadrant 1). The top 0-5 cm layer sediment contained the highest [Hg] for all tailings sites except CB and MR, where [Hg] was highest at the 5-10 cm depth and 10-15cm depth, respectively (Figure 1).

Aquatic invertebrates from all tailings-affected wetlands were found to have significantly higher [Hg] than insects from reference sites ($p < 0.001$, Table 3, Figure 2). Average invertebrate [Hg] ranged from 0.76 ± 0.64 ppm at CB to 4.20 ± 2.33 ppm at OS, while invertebrates from reference sites had only 0.21 ± 0.21 ppm Hg at MB REF and 0.35 ± 0.25 ppm Hg at MR REF (Table 2). Average [Hg] in invertebrates from the two reference wetlands did not differ significantly between sites.

Invertebrate [Hg] was correlated with sediment [Hg] in the top 5cm of sediment, when all five tailings sites were analyzed together ($p < 0.001$). However, because OS sediment has exceptionally elevated [Hg], even for a tailings-affected wetland, analysis was also conducted without the OS site included. Without OS, there was no significant correlation between average invertebrate [Hg] and sediment [Hg] at either the quadrant level or the site level (sediment Hg from all quadrants averaged) within tailings sites.

Prey type (i.e. herbivore, omnivore, piercer or carnivore) had a significant influence on [Hg] of invertebrates. Within tailings sites, herbivores had significantly lower [Hg] than all other feeding groups ($p < 0.001$). However, there was no significant difference between omnivores, piercers, or carnivores.

Arsenic

Sediment [As] was significantly elevated at all sites ($p < 0.01$), and highest overall at MP; 17780.0 ± 15155.86 ppm (0-5 cm depth), 9861.8 ± 7687.0 ppm (5-10 cm depth) and 9327.1 ± 13253.5 ppm (10-15 cm depth). The highest sediment [As] was 31200 ppm, also from MP (quadrant 4). Average [As] was also highest in the top-most layer at all tailings sites except MR, where the highest [As] was found at the 10-15 cm depth (Figure 1). Water concentrations of total As ([TAs]) and dissolved As ([DAs]) were significantly elevated in water samples at all tailings sites, compared to both reference sites (Table 2). OS was found to have the highest As in water at 1200 ppb [TAs] and 410ppb [DAs]. Total [Hg] in water was below instrument detection limits (0.0001 ppm) at both tailings and reference sites, and therefore results were not included in Table 2.

Arsenic concentrations in aquatic invertebrates from tailings sites showed evidence of being elevated when compared to reference sites ($p < 0.0001$). However, much of the reference data was below method detection limits (5 of 6 samples), and no samples from MR were analyzed due to insufficient mass. Invertebrate [As] from tailings sites had a median of 160 ppm, while the one data point within MDL's was 8.8 ppm As (Table 3).

Dolomedes spiders (piercers) from three tailings sites (CB, MP & OS) had significantly lower [As] than *sympetrum* dragonfly nymphs, which consume their prey whole ($p < 0.05$). Instead, piercers had similar average [As] to the omnivore genus *hyellella*, which feed on algae and detritus (Figure 3).

DISCUSSION

Wetland sediment analysis revealed that four of the five tailings-affected sites had elevated [Hg] compared to reference sites, and that all five tailings sites had

concentrations above the sediment Hg PELs set by the CCME. The most elevated sample (140 ppm Hg), which was found at the OS wetland in the top 0-5 cm of sediment, was over 820x the CCME sediment quality guideline level (ISQG), and over 285x the PEL (CCME, 2014). The top 5 cm of sediment contained the highest [Hg] and [As] at most of the sites, increasing the risk of exposure for many species including aquatic invertebrates, which often burrow into wetland sediment. This trend may be, in part, a result of low plant productivity (and therefore a lack of deposited organic matter) at impacted wetlands. Visual observations and photographic comparisons in the field also confirmed relatively low plant abundance at tailings-impacted wetlands compared to reference wetlands.

Similarly, elevated [As] was found in sediment at all five tailings-affected wetlands compared to sediment at reference sites, with concentrations surpassing CCME guidelines at all sites. Sediment samples from MP were exceptionally high (up to 31200 ppm As) and surpassed the ISQL by over 5280x and PEL by over 1830x (CCME, 2014). Average [As] found at the two reference sites also surpassed the PELs for [As] in sediment, although to a far lesser degree (5x for MR REF, 4x for MB REF). This is likely due to elevated background [As] in bedrock units of the Meguma Supergroup associated with the gold deposits in Nova Scotia (Kontak & Jackson, 1999; Parsons et al., 2012, Kennedy and Drage, 2016). Water [As] also surpassed CCME guidelines at all tailings sites. The most elevated water samples were at OS, where DAs was 410ppb and TAs 1200 ppb; 240x CCME water quality guidelines (Table 2). Invertebrate [As], showed some evidence of being elevated at OS and MP sites. MP invertebrates had the highest median [As] at 650 ppm.

Aquatic invertebrates from all five tailings sites had elevated [Hg] compared to reference specimens (Table 3). Elevated [Hg] in invertebrates was even found at MR, despite the fact that sediment [Hg] at MR was not statistically elevated compared to reference data (although it did still surpass PELs). This demonstrates that even tailings sites with lower Hg contamination may still pose a Hg bioaccumulation risk to the species living at or near them. Invertebrates from OS had particularly elevated [Hg] at 4.20 ± 2.33 ppm; over 12x the average [Hg] found in reference organisms. Bioaccumulation of [Hg] in invertebrates at this OS site has only been studied once before to our knowledge, in an undergraduate honours thesis project (Robinson et al., 2015). Dragonfly larvae, damselfly larvae, and aquatic spiders collected from the same OS wetland were found to have up to 2.0ppm [Hg] compared to reference samples (up to 0.24 ppm Hg) (Robinson, 2016, unpublished). Our results show that [Hg] in invertebrates at OS is even higher than previously demonstrated by this initial study.

Invertebrate [Hg] did not differ between quadrants at tailings sites, likely due to the mobility of many aquatic invertebrate species. There was also no significant difference in average invertebrate [Hg] between tailings sites, with the exception of OS, where significantly higher average [Hg] was found in invertebrates compared to organisms at other tailings sites. The fact that OS had both the highest sediment [Hg] and the highest invertebrate [Hg] suggests that they may be correlated.

Aquatic macroinvertebrates are often categorized based on their feeding habits: shredders, collectors, grazers (or scrapers) and predators (Anderson & Cummins, 1979). However, the extensive and intricate food web interactions of individual aquatic invertebrate species are complex and can differ by life stage, size, and environment.

Therefore, we categorized invertebrates in a more simplified method as herbivores, omnivores, carnivores, and piercers (those which pierce and digest the inside of their prey without consuming the whole prey). Herbivores had significantly lower [Hg] than all other feeding groups, with no significant difference in [Hg] found between omnivores, piercers, or carnivores.

Dolomedes spiders (piercers which feed on aquatic prey) from tailings sites did show evidence of having lower [As] than carnivores which feed on their prey whole (genus *Sympetrum* and *Aeshna*) (Figure 3). This may be due to the fact that adsorption of As into invertebrates exoskeletons seems to be a consistent and significant mechanism (Mason et al., 2000; Lavilla et al., 2010). Lavilla et al. (2010) found that up to 98% of As in *anisoptera* species was bound to their exoskeletons. Exoskeletons would not be consumed by piercers, but would be by carnivores which feed on their prey whole.

Here we have demonstrated that sediment and water remain highly contaminated at historical gold mine tailings sites, far surpassing CCME guidelines in many instances. We have also shown that aquatic invertebrates at these sites are elevated in [Hg], and possibly in [As]. This represents a potential risk to the species who prey on aquatic invertebrates. In Nova Scotia, predators of aquatic invertebrates and emergent adult insects include a number of species at risk such as the little brown bat, wood turtle, chimney swift, and the rusty blackbird. All of these species are known to feed at wetland habitat, increasing their risk of exposure (MTRI, 2008). Species of economic value, such as the Atlantic salmon, and trout species, along with other fish popular in the sportfishing industry, also live near tailings-impacted habitats and feed on aquatic invertebrates. This may present human health implications for people fishing near or downstream of tailings-

impacted waterbodies. More research assessing the transfer of Hg and As via aquatic and emergent invertebrates to other species and/or ecosystems would help to clarify these risks and guide long-term management decisions for these historical tailings sites.

CHAPTER 2 TABLES

Table 1. Coordinates for contaminated and reference sampling locations.

Data Collection Site	Site Code	Gold District	Lat, Long
Mitchell Brook Wetland	OS	Montague	44.712957, -63.518078
Muddy Pond	MP	Waverley	44.787272, -63.609547
Lake Catcha	LC	Lake Catcha	44.735497, -63.197402
Long Lake	CB	Caribou	45.053205, -62.938076
New Dam Flowage River	MR	Moose River	44.978545, -62.943813
Mitchell Brook Upstream (Reference)	MB REF	Montague	44.711236, -63.516126
New Dam Flowage River Upstream (Reference)	MR REF	Moose River	44.996520, -62.947748

Table 2. [Hg] and [As] in top 5cm of wetland sediment (ppm) and total ([TAs]) and dissolved arsenic ([DAs]) in water samples (ppb).

Collection Site/ Guideline	Sediment THg (ppm)			Sediment TAs (ppm)			Water TAs (ppb)	
	n	Mean ± SD	Median (IQR)	n	Mean ± SD	Median (IQR)	[TAs] (Dup.)	[DAs] (Dup.)
OS	4	92.50 ± 55.00	105.00 (67.50)	4	742.35 ± 975.44	184.70 (778.25)	1200	410
MP	4	10.86 ± 6.50	10.14 (4.56)	4	17780.05 ± 15155.86	17850.35 (25869.08)	190	160
CB	4	5.01 ± 0.6	4.70 (0.66)	4	8376.85 ± 1163.27	8728.70 (810.50)	150 (150)	120 (120)
LC	4	4.09 ± 1.7	4.09 (2.46)	4	2517.28 ± 1329.77	2900.65 (1491.08)	37	30
MR	4	0.81 ± 0.10	0.83 (0.07)	4	1427.70 ± 414.35	1498.90 (394.45)	12	9.5
MR REF	2	0.08 ± 0.01	0.08	2	88.80 ± 77.07	88.80	9.5	8
MB REF	2	0.39 ± 0.00	0.39	2	67.50 ± 4.24	67.50	1	<1.0
CCME ISQG		0.17			59		-	-
CCME PEL		0.486			17		-	-
CCME WQG		-			-		5	-
USEPA		-			-			0.34 (acute), 0.150 (chronic)

For reference: CCME ISQG, PEL, WQG — Canadian Council of Ministers of the Environment Sediment Quality Guidelines (ISQL), Probable Effects Levels (PEL), Freshwater Water Quality Guideline(WQG) for the protection of aquatic life (CCME, 2007a). USEPA – US Environ. Protection Agency.

Table 3. Mean [THg], mean and median [TAs] in aquatic invertebrates at historical tailings and reference sites. All data points below MDL's (n=17) were removed. n = number of composite samples analysed, indiv = number of individuals.

Site	[THg] (ppm, dw)		[TAs] (ppm, dw)		
	n (indiv.)	Mean \pm SD	n (indiv.)	Mean \pm SD	Median \pm IQR
OS	113 (946)	4.20 \pm 2.3 (n=946)	4 (34)	427 \pm 317.5	325 \pm 217.5
MP	156 (1181)	1.00 \pm 1.0 (n=1181)	6 (265)	420 \pm 257.0	380 \pm 457.5
CB	148 (1856)	0.76 \pm 0.6 (n=1856)	11 (262)	115.2 \pm 111.2	78 \pm 77
LC	156 (2020)	0.95 \pm 0.7 (n=2020)	6 (39)	136.7 \pm 90.2	160 \pm 120.3
MR	88 (734)	0.85 \pm 0.8 (n=734)	-	-	-
MR REF	45 (796)	0.35 \pm 0.3 (n=796)	1 (2)	8.8	8.8
MB REF	63 (706)	0.21 \pm 0.2 (n=706)	-	-	-

Table 4. Water chemistry data collected from tailings-impacted and reference sites, June-August, 2017. Data for water temp (Temp), dissolved oxygen (DO), specific conductance (SPC), total dissolved solids (TDS), and pH are provided, along with the number of samples (n) taken at each site.

Site	n	Temp (°C)	DO (%)	DO (mg/L)	SPC	TDS	pH
OS	4	25.75 \pm 1.77	85.50 \pm 11.79	6.90 \pm 0.80	109.83 \pm 41.33	71.50 \pm 26.95	7.40 \pm 0.52
MP	4	23.05 \pm 0.69	70.75 \pm 3.20	6.08 \pm 0.25	204.63 \pm 0.81	132.93 \pm 0.38	7.54 \pm 0.26
CB	4	23.57 \pm 0.09	88.75 \pm 3.59	7.50 \pm 0.29	25.80 \pm 2.21	16.74 \pm 1.44	7.61 \pm 0.25
LC	4	21.97 \pm 0.09	76.25 \pm 7.18	6.63 \pm 0.66	41.00 \pm 1.96	26.81 \pm 1.23	7.79 \pm 0.37
MR	4	20.52 \pm 0.05	96.50 \pm 0.58	8.68 \pm 0.10	23.65 \pm 0.06	15.85 \pm 0.50	6.23 \pm 0.45
MR REF	2	23.80 \pm 0.00	85.00 \pm 4.24	7.15 \pm 0.35	20.05 \pm 0.07	13.00 \pm 0.00	6.14 \pm 0.33
MB REF	2	24.25 \pm 0.21	61.50 \pm 2.12	5.15 \pm 0.21	342.10 \pm 0.28	220.00 \pm 3.25	7.35 \pm 0.21

CHAPTER 2 FIGURES

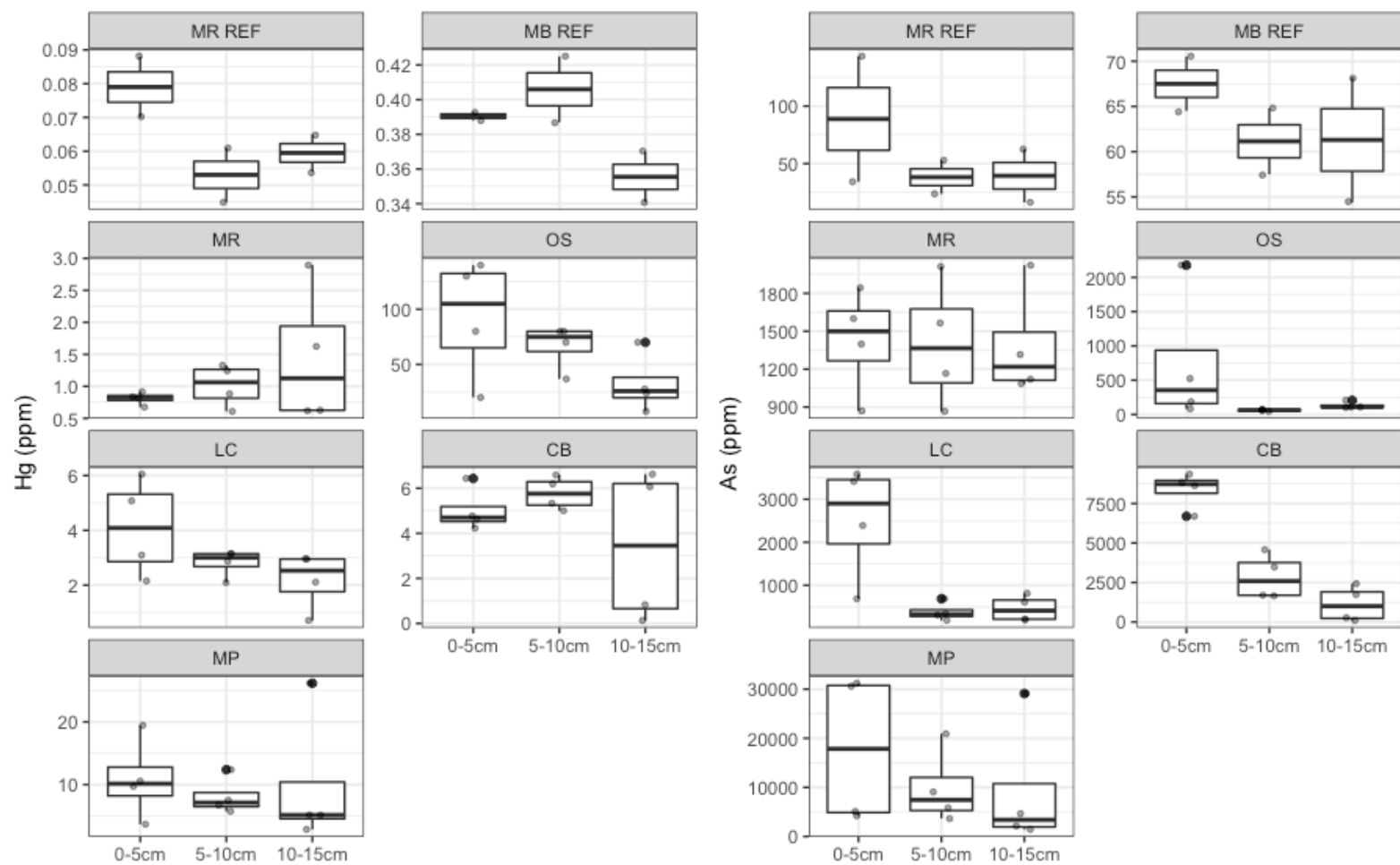


Figure 1. Sediment [Hg] and [As] at 0-5cm, 5-10cm and 10-15cm depths at tailings sites (n=4 quadrants) and reference sites (n=2 quadrants). Note that y axis differs by site.

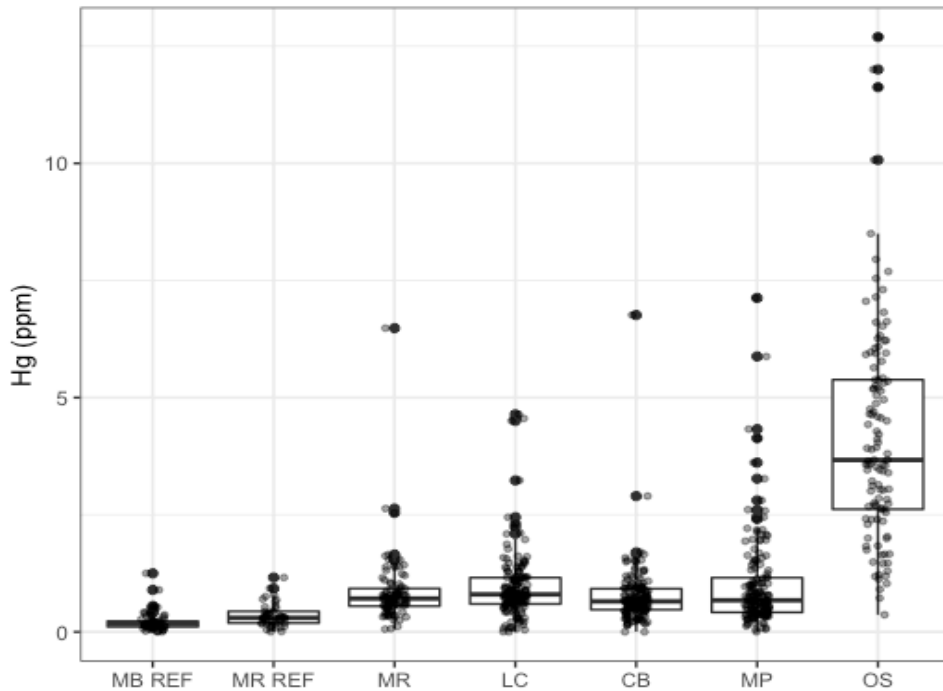


Figure 2. [Hg] (ppm, dry weight) in aquatic invertebrates from historical tailings and reference sites, pooled by sampling site.

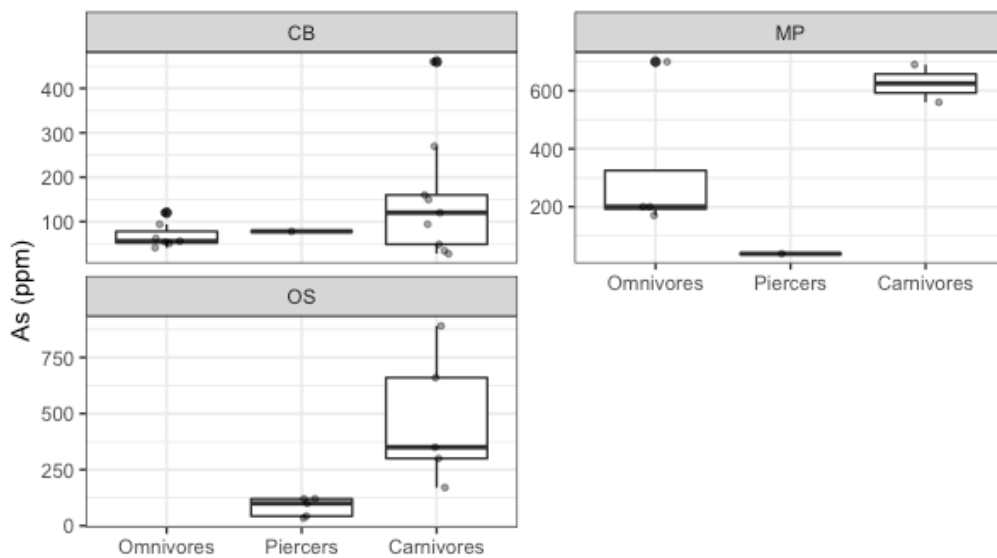


Figure 3. [As] (ppm, dw) among genera with three different feeding types; omnivores (*Hyelella*), piercers (*Dolomedes*), and carnivorous dragonfly larvae (*Sympetrum*).

CHAPTER 2 SUPPLEMENTAL INFORMATION

Taxonomic groups were analyzed separately for their own correlations with sediment [Hg] at both the quadrant level and the site level in order to conduct a preliminary evaluation of potential bioindicator species. Analysis was conducted on taxon of aquatic invertebrates which had ten or more composite samples. This included 26 genera of 24 families, 15 orders, 6 classes. Analysis on the level of order and family did not lead to any significant correlations with sediment [Hg]. However, on the level of genus, two groups were found to be significantly correlated with [Hg] in sediment. OS was not included in the analysis, as neither of these species were found at OS. These included a genus of mayfly, *Caenis* (order Ephemeroptera, family *Caenidae*), and a genus of snail, *Planorbula* (order Hygrophila, family *Planorbidae*). These two genera may have potential as future indicator species for Hg sediment contamination at tailings sites (Figure 4). There was insufficient data to assess correlations between invertebrate [As] and sediment [As].

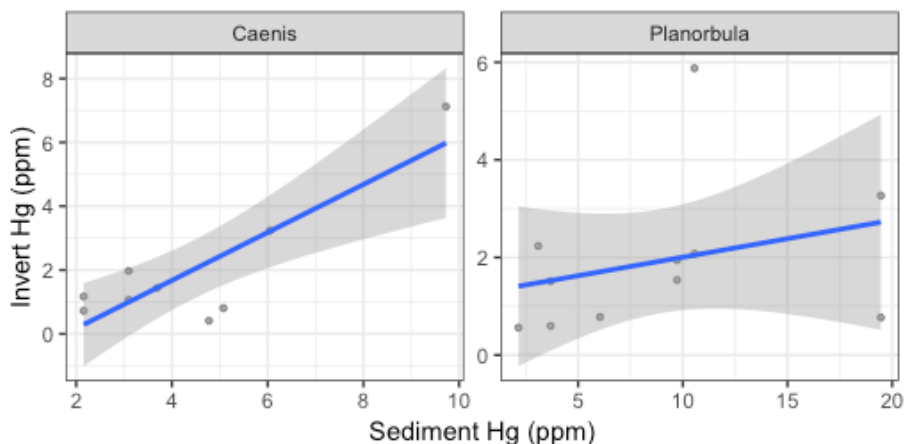


Figure 4. Two genera of aquatic invertebrates which may have potential as bioindicators of contaminated tailings sites. *Caenis* (n=9 composite samples) a genus of mayfly larvae (order Ephemeroptera, family *Caenidae*), and *Planorbula* (n=11 composite samples), a freshwater snail (order Hygrophila, family *Planorbidae*).

Table 5. Loss-on-ignition (LOI), total carbon (TC), organic carbon (OC), and calculated inorganic carbon (IC) in sediment core samples. Data is provided for three depths; 0-5cm (A), 5-10cm (B) and 10-15cm (C). Carbon analysis was not conducted for MR or reference sites.

Dist	Quad	LOI			TC			OC			IC *		
		A	B	C	A	B	C	A	B	C	A	B	C
CB	1	4.11	3.04	3.77	3.57	4.40	46.75	3.33	4.40	46.75	0.25	0.00	0.00
CB	2	10.42	2.36	4.39	1.74	9.00	42.57	1.56	8.25	42.57	0.18	0.75	0.00
CB	3	5.99	2.65	14.59	14.24	5.70	47.06	13.65	5.27	47.06	0.59	0.43	0.00
CB	4	11.97	4.20	12.66	10.40	50.02	52.16	9.74	50.02	52.06	0.66	0.00	0.10
LC	1	11.62	11.05	7.12	23.46	1.75	1.50	15.14	1.45	1.21	8.32	0.30	0.29
LC	2	8.78	5.68	4.53	22.85	2.29	1.78	16.43	2.12	1.78	6.42	0.17	0.00
LC	3	24.66	3.35	2.43	4.65	2.22	2.35	2.73	2.07	1.85	1.92	0.14	0.50
LC	4	54.20	5.85	5.84	39.85	17.88	7.75	39.85	12.34	4.19	0.00	5.54	3.56
MP	1	9.79	0.28	1.31	0.42	2.72	0.44	0.08	2.35	0.06	0.34	0.37	0.38
MP	2	5.65	3.41	1.92	4.10	0.45	0.45	2.95	0.07	0.05	1.15	0.38	0.40
MP	3	4.14	9.65	5.94	2.68	0.57	0.50	1.85	0.11	0.09	0.83	0.46	0.41
MP	4	11.66	6.65	1.44	0.60	1.48	0.54	0.05	0.74	0.10	0.55	0.74	0.44
OS	1	28.84	5.87	63.65	17.26	1.20	1.02	14.82	1.07	1.02	2.44	0.13	0.00
OS	2	6.55	5.29	76.75	7.07	0.61	1.62	6.01	0.44	1.18	1.06	0.17	0.44
OS	3	85.82	16.37	78.06	14.14	1.48	0.44	9.89	1.13	0.30	4.25	0.35	0.14
OS	4	76.41	43.41	78.68	7.41	0.54	0.32	5.91	0.38	0.24	1.49	0.15	0.08
MR	1	1.93	2.25	1.24	-	-	-	-	-	-	-	-	-
MR	2	2.94	1.68	1.20	-	-	-	-	-	-	-	-	-
MR	3	1.41	1.49	1.16	-	-	-	-	-	-	-	-	-
MR	4	2.03	1.30	1.40	-	-	-	-	-	-	-	-	-
MB REF	1	56.55	54.95	46.60	-	-	-	-	-	-	-	-	-
MB REF	2	57.01	54.15	51.88	-	-	-	-	-	-	-	-	-
MR REF	1	2.42	1.07	1.47	-	-	-	-	-	-	-	-	-
MR REF	2	2.25	12.28	1.96	-	-	-	-	-	-	-	-	-

*IC was calculated by subtracting OC from TC values.



Figure 5. Typical quadrant layout (A), and adapted layout (B) at “Old Stamp Mill” wetland site.

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Chapter 3. Emergent Invertebrates as Contaminant Biovectors at Historical Gold Mine Sites

ABSTRACT

Emergent insects have been shown to act as biovectors of contaminants from aquatic environments. In this study we examine mercury (Hg) and arsenic (As) accumulation in aquatic nymph and emergent adult stages for insect species from 3 orders (Ephemeroptera, Odonata, Plecoptera) from historical gold mine tailings-impacted wetlands. Nova Scotia's historical gold mining industry (1846-1940's) led to an estimated 3 million tonnes of mine tailings with elevated Hg and As, which were produced and deposited around the province where they still remain today, often overlapping with wetland habitat. The ecological effects of these tailings on Nova Scotia's aquatic environments remains largely unquantified to date. We found that insects are accumulating significant concentrations of contaminants during their aquatic life stage. Larvae from 5 tailings-impacted sites had an average [Hg] of 1.47 ± 1.73 ppm, and average [As] of 277.5 ± 244.12 ppm, compared to 0.26 ± 0.22 ppm [Hg] and 12.65 ± 5.28 ppm [As] at 2 reference sites. Hg concentration in larvae was positively correlated with average sediment [Hg] ($p < 0.0001$) while [As] in larvae was not. Exoskeleton casings shed during the emergent process were also collected and contained low average [Hg], but high average [As]. Casings from tailings sites were estimated to account for an average Hg loss of 14% and As loss of 93% through the shedding process. Adult emergent insects are potential biovectors of Hg; adults from tailings sites had an average [Hg] of 0.89 ± 0.91 ppm compared to 0.36 ± 0.45 ppm in reference adults.

INTRODUCTION

Gold mining in Nova Scotia has been a significant part of the development of the province, with over 1,200,000 troy oz of gold produced between 1861 and the mid-1940s. However, this era of mining also produced an estimated 3,000,000 tonnes of highly contaminated, waste tailing material, which still resides in untreated deposits throughout the province (Parsons et al., 2012). These tailing deposits often overlap with wetland habitat, as historical ore processing facilities required a freshwater source, and therefore, were typically built near lakes, streams, or rivers. Tailings and sediment samples from these mine sites contain up to 310,000 ppm arsenic ([As]), and 350 ppm mercury ([Hg]); far surpassing CCME sediment and soil quality guidelines (CCME; 2003; Parsons et al., 2012). Despite potential risks to human health and surrounding ecosystems, bioaccumulation of Hg and As in aquatic organisms and food webs near legacy gold mine tailings sites in Nova Scotia have generally not been characterized in much detail (Chapter 1).

Aquatic invertebrates can provide information on bioavailability and bioaccumulation of contaminants, as they are often exposed to contaminants in their immediate environment (water, sediment, food). In addition, they do not move great distances, and are relatively easy to collect. Many species of insects including those from Orders Odonata, Ephemeroptera and Plecoptera, are aquatic in their larval stage, then hatch from the water in their adult stage, often shedding their exoskeleton casing in the process. Numerous studies have found that emergent insects are a main source of prey biomass, carbon and nitrogen for terrestrial species feeding near shorelines or streambeds, including adult odonates, beetles, spiders, lizards, salamanders, birds and bats (Baxter et al., 2005; Henschel et al. 2001; Iwata et al. 2003; Sanzone et al., 2003). Predators have

been shown to depend on aquatic-sourced insects even hundreds of metres from the aquatic habitat (Power & Rainey, 2000; Raikow et al., 2011).

Emergent insects can act as biovectors of contaminants. In their aquatic larval stage, many invertebrates live in direct contact with sediment (often burrowing for periods of time) and water, making them especially susceptible to bioaccumulation of aquatic contaminants (Menzie, 1980). Emergent insects have been shown to act as biovectors of PCBs (Baxter et al., 2005; Maul et al., 2006; Raikow et al., 2011; Walters et al., 2008), As (Blais et al., 2007; Mogren et al., 2013; Torres & Johnson, 2001), Hg and MeHg (Jackson et al., 2011, Speir et al., 2014). Even in cases where individual emergent insects do not contain high contaminant concentrations individually, mass emergences can result in biomagnification in predators from adjacent terrestrial habitats (Blais et al., 2007; Mogren et al., 2013). Here, we evaluate the extent to which emergent insects may be accumulating and transferring contaminants from wetlands associated with legacy gold mine tailing sites.

METHODS

Sample collection took place throughout the growing seasons (May-August) of 2016 and 2017. Sites were selected from districts ranked among the 25 historically most productive sites in Nova Scotia. Five contaminated wetland/aquatic sites and two reference sites (Table 1) were selected based on wetland habitat, accessibility, and on historical mining production data for the contaminated sites (Parsons et al., 2012). The two reference sites were located upstream of the Montague and Moose River tailings sites; Mitchell Brook and New Dam Flowage River,

respectively. From here on, we use abbreviations to describe each site, which can be found in Table 1.

Aquatic invertebrates were collected using a kicknet during the summer months of 2016 and 2017, from four adjoining 4.57 x 4.57 m quadrants, placed alongside the shoreline of the wetland sites. At reference locations, greater quantities of invertebrates were found, therefore only two quadrants were used to conserve sampling time. At the Old Stamp Mill site, insufficient aquatic invertebrates were found, and therefore a larger quadrant size of 15 x 15 m was used, in order to collect enough sample mass for analysis.

Emergent invertebrates and empty casings were collected during July to September of 2017. We constructed floating Malaise-style traps in 2017 based on the successful design plans for similar studies (Tweedy et al., 2013; Jones et al., 2013). Two traps were placed at each site in the same locations as the aquatic invertebrates were collected. As emergent hatching has been shown to decline exponentially with distance from the shoreline (Baxter et al., 2005), the traps were placed near the shore or streambed, in vegetated areas. Traps were set out for 48-hour periods repeatedly over the course of 11 weeks, and all emergent insects and discarded casings found in the traps were collected.

Invertebrates were transported back to the lab on ice, left alive for a 24-hour gut purge, and washed with squirt bottle with RO water (US EPA, 2000; MOECC, 2006). They were also visually inspected for sediment particulates under a microscope, before being frozen at -20°C until analysis. Prior to analysis, all samples were thawed, and weighed to collect wet weight data. They were then dried overnight at a low temperature (50-55 °C, Schmidt et al., 2013), and weighed again in order to calculate moisture content.

Samples were identified to genus (when possible) using identification guides (Clifford, 1991; Paulson, 2011; Voshell, 2002). Emergent invertebrate larvae samples collected from tailings wetlands (n=276) and reference wetlands (n=43) were composed of 30 genera from 3 orders; Ephemeroptera (n=47), Odonata (n=258), and Plecoptera (n=14). Casings and emergent adults from these three orders were collected from traps at tailings sites (n=131 casings, n=176 adults) and reference wetlands (n=15 casings, n=78 adults). Due to the large quantity of samples, aquatic invertebrates from the same genus and site were pooled as composite samples and homogenized to a fine powder using a Retsch mixing mill. The only exception were samples with very low mass, which were left whole in order to avoid loss of sample material. All casings and adult samples were analyzed separately and individually.

[THg] was analyzed in all invertebrate samples using a Milestone DMA 80.3 in a clean-room laboratory at Saint Mary's University. Trace-element protocols were followed to ensure no cross contamination of samples occurred. Samples were run in cleaned quartz sample boats. Each analysis run was preceded with multiple blanks, a series of reference liquid mercury standards (0, 5, 10, 15, and 20 ppm), along with 2-3 certified reference materials (CRM), to ensure calibration accuracy. CRM's used for invertebrate runs included TORT-3, DORM-4 and DOLT-5, all of which were found to be in normal range. Each sample was placed in an acid-washed, quartz sample boat, weighed and recorded on the DMA80 computer software. To prevent contamination carry-over, two blanks were run between invertebrate samples from differing quadrants, and a full set of blanks were run between locations. All [Hg] data are presented as ppm dry weight (dw).

During analysis, there was no major difference found between genera, family, or order in terms of Hg or As accumulation, in either the larval stage or adult life stages. Therefore, for the majority of the analysis, orders are combined. However, taxonomic variation in [Hg] and [As] can be found in the Supplemental Material.

A subset of Odonata samples from two genera (*sympetrum* and *aeshna*) were sent for As analysis, which included 22 composite larvae samples (n= 211), 16 casings and 17 adult insects from tailings sites and 4 composite larvae samples, 4 casings, and 5 adults from reference sites. During As analyses, a number of samples (n=24) were reported to have [As] below method detection limits (MDL) as a result of having low mass, with MDL's varying widely from 8.8 to 660ppm. All [As] data is presented as ppm of dry weight.

Water samples were collected in October 2017 using the “clean-hands, dirty hands” technique to avoid external contamination. Sub-samples for total mercury were preserved using ultrapure nitric acid in Teflon bottles, immediately following collection. Water was analyzed for THg with Milestone DMA 80.3. Water samples were also sent to Analytical Services Unit, Queen's University where filtered (0.45 µm filter) and unfiltered samples were analyzed via ICP-MS. Blanks and controls were included with runs, with scandium, indium and bismuth used as internal standards.

Bulk sediment samples of the top 5cm of sediment were collected from all sites using a short PVC pipe sediment corer. Samples were kept on ice during transport back to the lab, where they were frozen immediately to preserve their chemistry. The samples were eventually thawed and dried at approximately 55 °C to avoid loss of Hg (Heiri et al., 2001), lightly disseminated using a mortar and pestle, and shaken through a 2 mm sieve (to remove excess organic matter).

To avoid equipment contamination in our laboratory by highly contaminated samples, all sediments were sent to Bureau Veritas laboratory for Hg, As, and additional element analyses via ICP-MS (method code *AQ250*; 1:1:1, HCl: HNO₃: H₂O, digestion ultratrace ICP-MS). A number of sample duplicates were run to ensure consistency, along with blanks, and standard materials (DS10 and OREAS4EA). Because some samples surpassed both Hg and As MDL's, 12 samples were re-analyzed using via ICP-ES (method code *AQ370*; Aqua Regia – ICP-ES).

Statistical analysis was conducted using RStudio. Log₁₀ transformations of Hg and As data removed heterogeneity of variance within both data sets prior to statistical analysis, however figures demonstrate actual values. Mercury data was found to be non-parametric, therefore the Kruskal-Wallis Test was used, along with the Pairwise Wilcoxon rank sum test to calculate comparisons between sites. Arsenic data was non-parametric and left-censored due to varying MDL's, therefore the Kaplan-Meier analysis was used.

Estimates of Hg and As lost via shedding of casings were calculated for sites where sufficient data was available using the following equations, where *dw* represents dry weight, and *X* represents the contaminant Hg or As:

$$(1) \quad \frac{X \left(\frac{mg}{kg} \right) \times dw (kg)}{N} = X (mg) \times 100000 = X (ng)$$

$$(2) \quad \frac{\text{Average Casing } X (ng)}{(\text{Average Adult } X (ng) + \text{Average Casing } X (ng))} \times 100 = \% X \text{ shed in casing}$$

RESULTS

Mercury

Sediments from tailings-affected wetlands were found to have significantly higher average [Hg] at all sites except for MR (See Table 1 for site abbreviations). The OS tailings wetland had the highest average [Hg] of 92.5 ± 55.0 ppm; over 230x the [Hg] found in reference site sediment, and over 190x PELs (Table 2). Water total [Hg] were below instrument detection limits (IDLs) of 0.0001 ppm at both tailings and reference sites, and therefore, were not included in Table 2.

Mercury in aquatic insect larvae from all five tailings wetlands had elevated [Hg] compared to larvae from reference wetlands ($p < 0.0001$; Table 3). Within tailings sites, there was a positive relationship between average sediment [Hg] and [Hg] in larvae [Hg] ($r_s = 0.42$, $p < .0001$). The OS tailing wetland, which had the highest sediment [Hg] (Table 2), also had the highest median [Hg] in larvae of 4.28 ppm (Table 3). When pooled, [Hg] in larvae from tailings sites ranged from 0.07 to 12.00 ppm while reference larvae ranged from 0.001 to 1.15 ppm (Figure 2).

Insect casings at tailings sites, shed during the hatching process, also had higher median [Hg] (0.13 ppm) than casings from reference sites (0.05 ppm). Average [Hg] in casings was positively correlated with average sediment [Hg], but with a steeper curve than found for larvae ($r_s = 0.56$, $p < 0.0001$). Overall, casings had lower concentrations of [Hg] than both larvae and adults by a factor of 2.3x and 1.43x, respectively. The percent of Hg lost to shed casings was estimated for three tailings sites where sufficient data was available; 8.1% at CB, 9.7% at LC, and 23.7% at MP, for an average of 13.9 % overall.

Adult insects caught at tailings sites also had significantly higher median [Hg] than those from reference sites (Table 3). Tailings adults had [Hg] ranging from 0.0001 to 6.38 ppm (median = 0.89 ppm) while reference adults had [Hg] from 0.00004 to 3.41 ppm (median = 0.36 ppm). Adults from 4 of the 5 tailings sites had elevated [Hg] ($p < 0.05$) compared to both reference sites, with the exception of adults from MR, which were only elevated compared to one of the reference sites (MR REF). Overall, the number of adults caught differed greatly by site, with the most productive sites being MR ($n=84$), followed by the MR REF site ($n=69$), and the least productive being the OS tailings site ($n=3$). There was, once again, a positive correlation between average sediment [Hg] and adult insect [Hg] within tailings wetlands ($r_s=0.41$, $p < 0.0001$).

Arsenic

Sediment [As] was also elevated in sediment from all tailings sites, with MP sites found to have the highest [As] at 17780 ± 1516 ppm; over 200x that of reference sediment, and over 1000x PELs (Table 2). Water concentrations of total As and dissolved As ([DAs]) were significantly elevated in water samples from all tailings sites. OS was found to have the highest [As] at 1200 ppb, surpassing the CCME water quality guideline (WQG) by a factor of 240, and [DAs] at 410 ppb (Table 2).

Odonate larvae from the OS and MP tailings sites also had significantly higher [As] than larvae at reference wetlands ($p < 0.001$; Table 4). However, at LC, CB and MR tailings sites, average [As] in the larvae was not significantly higher than in reference specimens. However, this may have been affected by the estimation of [As], as described in the methods. Overall, larvae [As] were not positively correlated with average sediment [As] at tailings sites. [As] in

larvae from tailings sites was estimated to range from 17 to 890 ppm, and when pooled, were found to have significantly higher average [As] than pooled reference larvae ($p < 0.01$; Table 3) which were estimated to range from 8.8 to 20.0 ppm.

Odonate casings from tailings sites analysed for As ($n=14$), in contrast, has extremely elevated [As] which surpassed larvae and adult average [As] by factors of 11.9x and 71.9x, respectively, indicating that much of the As is likely shed in the hatching process. The amount of As lost to shed casings was estimated for two tailings sites with sufficient data; 89.5% at CB, and 96.5% at MP, for an average of 93.0% lost. Similar to larvae, casings were not significantly correlated with average sediment [As] at tailings sites. Casings from reference sites were all below the DL of 130 ppm, which were high as a result of the low weights (Table 4). The majority of adult Odonates at tailings sites and all samples from reference sites had As below MDL's.

DISCUSSION

Emergent insects are among the key sources of energy and carbon for both aquatic and terrestrial species including; fish, adult Odonates, beetles, spiders, salamanders, bats, and birds (Baxter et al., 2005). In Nova Scotia, potential predators on emergent insects include a number of species at risk such as the little brown bat, wood turtle, chimney swift, and the rusty blackbird, which all commonly feed on wetland aquatic and emergent insects (Environment & Climate Change Canada, 2015). Key predators also include species of significant economic and social value, such as the Atlantic salmon, brook trout and other fish popular in the fishing industry, and key migrant bird species including flycatchers and warblers.

Although these legacy gold mine tailings deposits have led to some of the most contaminated wetlands in the province, until now little work has been done to assess their ecological impact to species nearby. Here we show that Hg and As-contaminated wetland sediment leads to elevated [Hg] and [As] in aquatic insects. In line with previous research elsewhere, our study has demonstrated the potential of emergent insects to transfer Hg, and to a limited extent As, from contaminated tailing-impacted wetlands in Nova Scotia.

In particular, emergent insects from impacted wetlands have been shown to be especially effective in transporting Hg out of the wetlands. All five tailings sites demonstrated elevated Hg in larvae, which was positively correlated to average [Hg] in sediment. To contextualize the results, in a study conducted at Kejimikujik National Park, NS (a nationally documented Hg hotspot due to atmospheric Hg deposition) Odonate nymphs were found to have mean [Hg] of 0.25 ± 0.11 ppm (Buckland-Nicks et al., 2014). Average [Hg] in larvae from the historical tailings sites were far higher than the maximum [Hg] values for Kejimikujik sites, and ranged from 0.70 ± 0.27 (CB Site) to 4.70 ± 2.23 ppm (OS Site), representing the highest aquatic insect [Hg] documented thus far in the province for aquatic invertebrates. Those extraordinarily elevated concentrations in aquatic insects may pose a threat to aquatic species (i.e. fish) feeding on insects from these sites, and indirectly to humans and other piscivorous predators downstream of these contaminated wetlands.

A significant amount of the Hg burden in larvae was retained in adult insects after hatching, with a relatively low amount being shed in the discarded casings. This general trend of low Hg concentrations found in casings compared to larvae and adults is consistent with other studies in Nova Scotia (Buckland-Nicks et al., 2004). This may mean predators of adult emergent

insects, such as bats, birds, amphibians and spiders, are also at risk of increased Hg accumulation. Other studies have shown that in areas with aquatic Hg contamination, terrestrial-feeding birds may be at even greater risk than aquatic-feeding birds (Baxter et al., 2005). Studies have shown emergent insects have the ability to transfer sediment-sourced contamination long distances. In one study, sediment PCBs were transferred via emergent insects to spiders up to 5 m away from the aquatic source, and to social wasps, which also feed on emergent insects, up to 30 m inland (Raikow et al., 2011).

Methylmercury (MeHg) is highly toxic to living organisms and commonly biomagnifies in food webs. MeHg results from methylation of inorganic Hg (Hg 2+) and often occurs in anaerobic environments with high microbial activity and organic carbon, such as wetlands. MeHg is both highly bioavailable, and slow to be eliminated from tissue, making it especially susceptible to bioaccumulation and biomagnification (O'Driscoll et al., 2005). As it is lipophilic, it is also able travel to vital organs, including the brain, and through the placental membrane barrier (CCME, 2003; Edmonds et al., 2010; Doe et al., 2017). In predacious insects and insectivorous fish, MeHg has been shown to account for the majority of THg (Mason et al., 2000). Buckland-Nicks et al. (2014) found that MeHg accounted for 91.5% of THg in Odonate nymphs and 82.9% of THg in adult Odonates. Therefore, the high concentrations of THg found in insects at tailings sites may also indicate elevated [MeHg] moving through local food webs. Using data from Buckland-Nicks et al. (2014), larvae from historical tailings sites could contain up to 4.30 MeHg and adults up to 3.76 MeHg (estimates for insects from OS site). This would far surpass the CCME MeHg guideline for the protection of aquatic life (0.033ppm ww) even after dry weight to wet weight conversions.

[As] was also found to be elevated in aquatic larvae, but only at tailings sites with exceptionally elevated [As] in wetland sediment: MP and OS. Unlike Hg, a large portion of As was found to be shed in casings during the hatching process, and therefore was not retained in the adult insects. This is, again, consistent with past literature which has shown that adsorption of As into an invertebrate's exoskeleton is a common mechanism for shedding arsenic (Mason et al., 2000). Lavilla et al. (2010) found that up to 98% of As in Anisoptera species were bound to their exoskeletons. However, [As] concentrations in emergent insects from highly contaminated sites still represent a potential risk for terrestrial and aquatic predators.

Predators which feed on the larval stage (ex. fish) will be at higher risk of As accumulation than those which feed on adults (ex. bats, birds). Exposure will also be greatly influenced by predator feeding mechanisms, as species which eat their prey whole will ingest the As-rich exoskeleton. However, spiders which feed by injecting digestive juices into their prey and do not consume the remaining exoskeleton, could be less exposed. Correspondingly, species which feed on spiders rather than species which consume whole-body insects will be less at risk. This was demonstrated in an in-lab experiment conducted by Mogren et al. (2013), where two terrestrial invertebrates (cobweb spider and praying mantis) were fed As-treated adult mosquitoes (*Culex tarsalis*) and evaluated for As bioaccumulation. In the cobweb spiders (*Tidarren haemorrhoidale*), there was no significant difference in As accumulation between spiders fed As-treated and non-treated mosquitoes. However, in the praying mantis species (*Tenodera aridifolia sinensis*) which eat their food whole, those fed As-treated mosquitoes accumulated significantly higher As (658 ppb vs. 145 ppb reference) (Mogren et al., 2013).

Moving forward, more work is needed to define the extent of the contamination from tailings deposits. Mercury, in particular, has been shown to travel downstream far distances from the original sources of contamination. Jackson et al. (2011) demonstrated that insectivorous birds up to 137 km downstream from historical Hg contamination still contained elevated blood Hg concentrations. These authors concluded that the large geographical spread of aquatic contamination into terrestrial habitats, "...greatly expands the scale of concern for those tasked with managing risk to wildlife near mercury-contaminated rivers" (Jackson et al., 2011). In addition, studies on the effects of contaminant accumulation on insect biomass are needed. Predator distribution and behavior is influenced by the number of emergent insects (Nakano & Murakami, 2001, Murakami & Nakano 2002; Power & Rainey, 2000; Power et al., 2004; Sweeney & Vannote, 1982), and as may be expected, experiments which manipulated emergent biomass affected the short-term behavior, growth and abundance of terrestrial predators along shorelines (Baxter et al., 2005; Kato et al. 2003; Sabo & Power, 2002). The effects of these tailings sites, not only in contaminant transfer, but in effects to insect biomass and biodiversity, are not well understood.

In conclusion, we have shown that the accumulation of Hg and As in aquatic insects, and the transfer of Hg via aquatic insects, is occurring at historical gold mine tailings sites in Nova Scotia. This represents a potential risk to both aquatic and terrestrial consumers, and may have implications for people fishing near these sites. The retention of Hg in adult insects hatching from these contaminated wetlands expands the spatial extent of the risks presented to terrestrial predators and to neighboring ecosystems. More work to assess the spatial extent of contamination

at tailings deposits, and confirmation of Hg accumulation in higher predators, would help to clarify the ongoing risks associated with these historical mine wastes.

CHAPTER 3 TABLES

Table 1. Data collection sites and coordinates.

Data Collection Site	Site Code	Gold District	Lat, Long
Old Stamp Mill Wetland	OS	Montague	44.712957, -63.518078
Muddy Pond	MP	Waverley	44.787272, -63.609547
Lake Catcha	LC	Lake Catcha	44.735497, -63.197402
Long Lake	CB	Caribou	45.053205, -62.938076
New Dam Flowage River	MR	Moose River	44.978545, -62.943813
Mitchell Brook Upstream (Reference)	MB REF	Montague	44.711236, -63.516126
New Dam Flowage River Upstream (Reference)	MR REF	Moose River	44.996520, -62.947748

Table 2. Average and median values for [Hg] and [As] in top 5cm of wetland sediment (ppm) and total ([TAs]) and dissolved arsenic ([DAs]) in water samples (ppb).

Collection Site/ Guideline	Sediment THg (ppm)			Sediment TAs (ppm)			Water TAs (ppb)	
	n	Mean ± SD	Median (IQR)	n	Mean ± SD	Median (IQR)	[TAs] (Dup.)	[DAs] (Dup.)
OS	4	92.50 ± 55.00	105.00 (67.50)	4	742.35 ± 975.44	184.70 (778.25)	1200	410
MP	4	10.86 ± 6.50	10.14 (4.56)	4	17780.05 ± 15155.86	17850.35 (25869.08)	190	160
CB	4	5.01 ± 0.6	4.70 (0.66)	4	8376.85 ± 1163.27	8728.70 (810.50)	150 (150)	120 (120)
LC	4	4.09 ± 1.7	4.09 (2.46)	4	2517.28 ± 1329.77	2900.65 (1491.08)	37	30
MR	4	0.81 ± 0.10	0.83 (0.07)	4	1427.70 ± 414.35	1498.90 (394.45)	12	9.5
MR REF	2	0.08 ± 0.01	0.08	2	88.80 ± 77.07	88.80	9.5	8
MB REF	2	0.39 ± 0.00	0.39	2	67.50 ± 4.24	67.50	1	<1.0
CCME ISQG		0.17			59		-	-
CCME PEL		0.486			17		-	-

<i>CCME WQG</i>	-	-	5	-
<i>USEPA</i>	-	-		0.34 (acute), 0.150 (chronic)

For reference: CCME ISQG, PEL, WQG — Canadian Council of Ministers of the Environment Sediment Quality Guidelines (ISQL), Probable Effects Levels (PEL), Freshwater Water Quality Guideline(WQG) for the protection of aquatic life (CCME, 2007a). USEPA – US Environ. Protection Agency.

Table 3. Emergent insect [Hg] (ppm, dry weight) by life stage from each data collection site.

Site Code	Larvae [Hg]			Casings [Hg]			Adults [Hg]		
	n samples (indiv.)	Mean ± SD	Median (IQR)	n	Mean ± SD	Median (IQR)	n	Mean ± SD	Median (IQR)
MB REF	22 (180)	0.16 ± 0.05	0.16 (0.05) ^a	6	0.04 ± 0.06	0.02 (0.04) ^{ab}	9	0.43 ± 0.33	0.39 (0.50) ^{ab}
MR REF	21 (363)	0.37 ± 0.28	0.32 (0.17) ^a	28	0.17 ± 0.17	0.10 (0.30) ^{ac}	69	0.36 ± 0.47	0.24 (0.16) ^a
MR	55 (340)	0.75 ± 0.42	0.67 (0.25) ^b	11	0.44 ± 0.50	0.32 (0.26) ^{acd}	85	0.50 ± 0.39	0.41 (0.24) ^b
CB	67 (718)	0.71 ± 0.28	0.65 (0.35) ^b	8	0.61 ± 1.10	0.21 (0.21) ^{cd}	19	0.87 ± 0.56	0.75 (0.54) ^c
MP	50 (471)	1.13 ± 1.13	0.80 (0.95) ^b	47	0.87 ± 0.77	0.61 (1.20) ^d	26	1.03 ± 1.05	0.78 (0.54) ^c
LC	65 (867)	0.98 ± 0.51	0.84 (0.43) ^b	87	0.14 ± 0.32	0.00 (0.10) ^b	47	1.30 ± 0.79	1.17 (1.00) ^d
OS	43 (232)	4.70 ± 2.23	4.28 (1.92) ^c	3	8.81 ± 1.67	8.17 (1.57) ^c	3	4.54 ± 2.79	5.90 (2.52) ^d
Reference Sites	43 (543)	0.26 ± 0.22	0.18 (0.17)	34	0.11 ± 0.14	0.05 (0.18)	79	0.36 ± 0.45	0.25 (0.22)
Tailings Sites Combined	280 (2628)	1.47 ± 1.73	0.80 (0.91)	156	0.62 ± 1.43	0.13 (0.59)	180	0.89 ± 0.91	0.63 (0.71)

Groups with different letters (^{a, b, c, d}) are significantly different from each other for that life stage (Pairwise Wilcoxon Ranked Sum Test; p < 0.05).

Table 4. Mean and median [As] (ppm, dry weight) in Odonates by life stage from tailings and reference sites. Kaplan-Meier analysis was used as data was non-parametric and left-censored. ND - indicates number of samples below As method detection limit.

Site Code	Larvae [TAs]				Casing [TAs]				Adult [TAs]			
	n (indiv.)	ND	Mean ± SD	Med.	n	ND	Mean ± SD	Med.	n	ND	Mean ± SD	Med.
MB REF	2 (41)	2	-	-	4	4	-	-	5	5	-	-
MR REF	2 (5)	1	8.8	8.8	-	-	-	-	-	-	-	-

CB	9 (89)	2	136.1 ± 149.9	49.0	3	0	3700 ± 848.5	3600	4	4	-	-
MP	2 (26)	-	625.0 ± 91.9	-	2	0	4075 ± 1969	3600	5	2	80 ± 85.7	45
LC	6 (10)		136.1 ± 149.9	180.0	5	0	2330 ± 1122	2800	5	5	-	-
OS	5 (40)	1	396.7	300.0	4	0	3700 ± 848.5	-	2	1	25	-
Reference Sites Combined	4 (46)	3	8.8	-	4	4	-	-	5	5	-	-
Tailings Sites Combined	22 (165)	4	235.4 ± 240.5	170	14	0	3325 ± 1532	3100	17	13	39.2 ± 52.9	-

CHAPTER 3 FIGURES

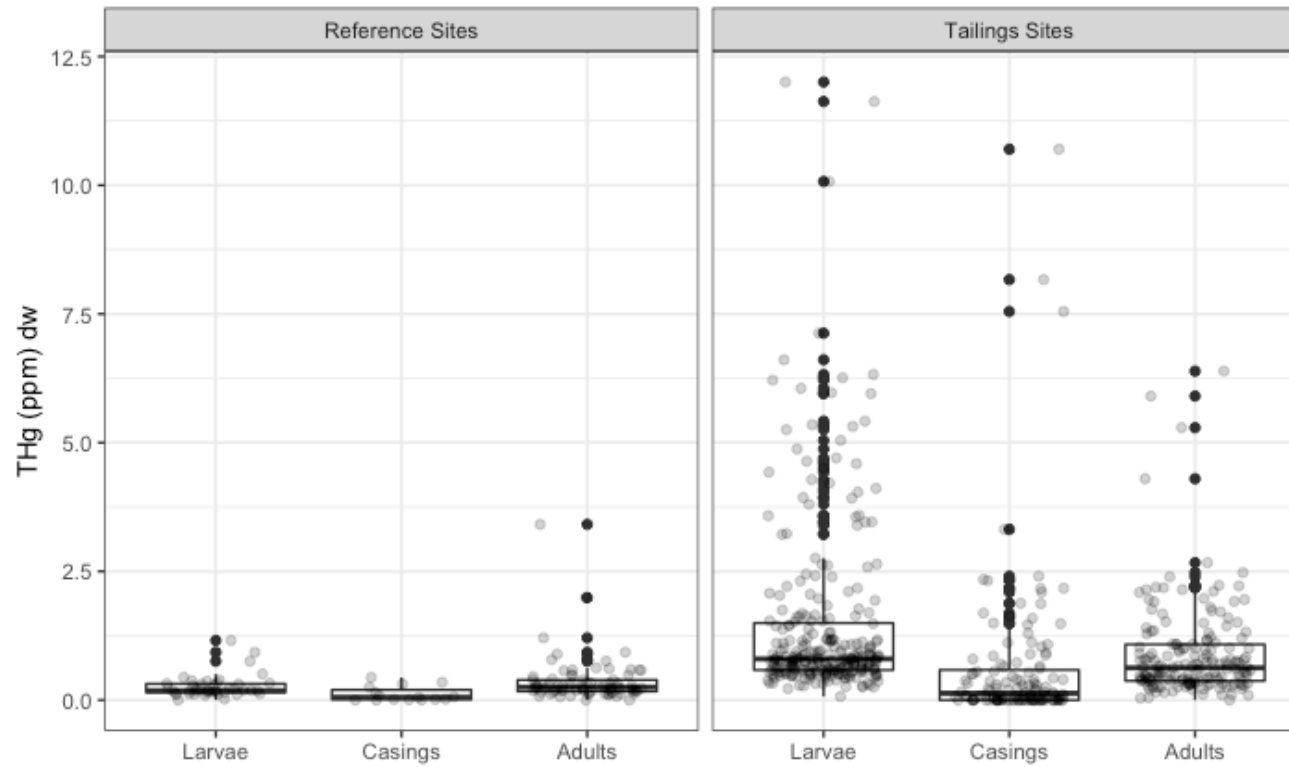


Figure 1. Summary of [Hg] and estimates of average [As] in larvae, casings, and adult emergent insects from historical tailings sites and reference sites.

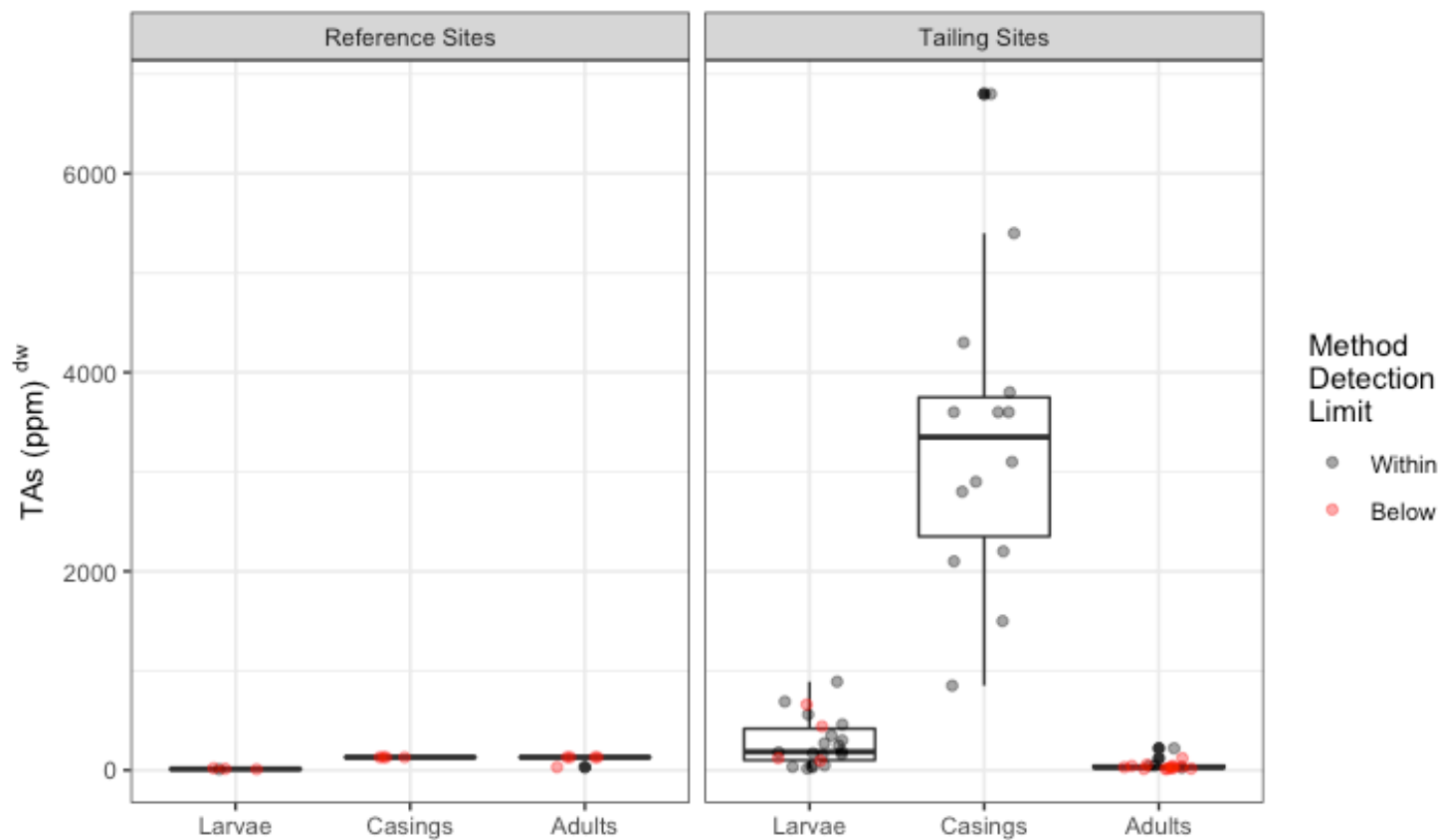


Figure 2. Summary of [Hg] and estimates of average [As] in larvae, casings, and adult emergent insects from historical tailings sites and reference sites. Data points below method detection limits (MDL) are in red, and are represented using the MDL value itself (i.e. max possible value).

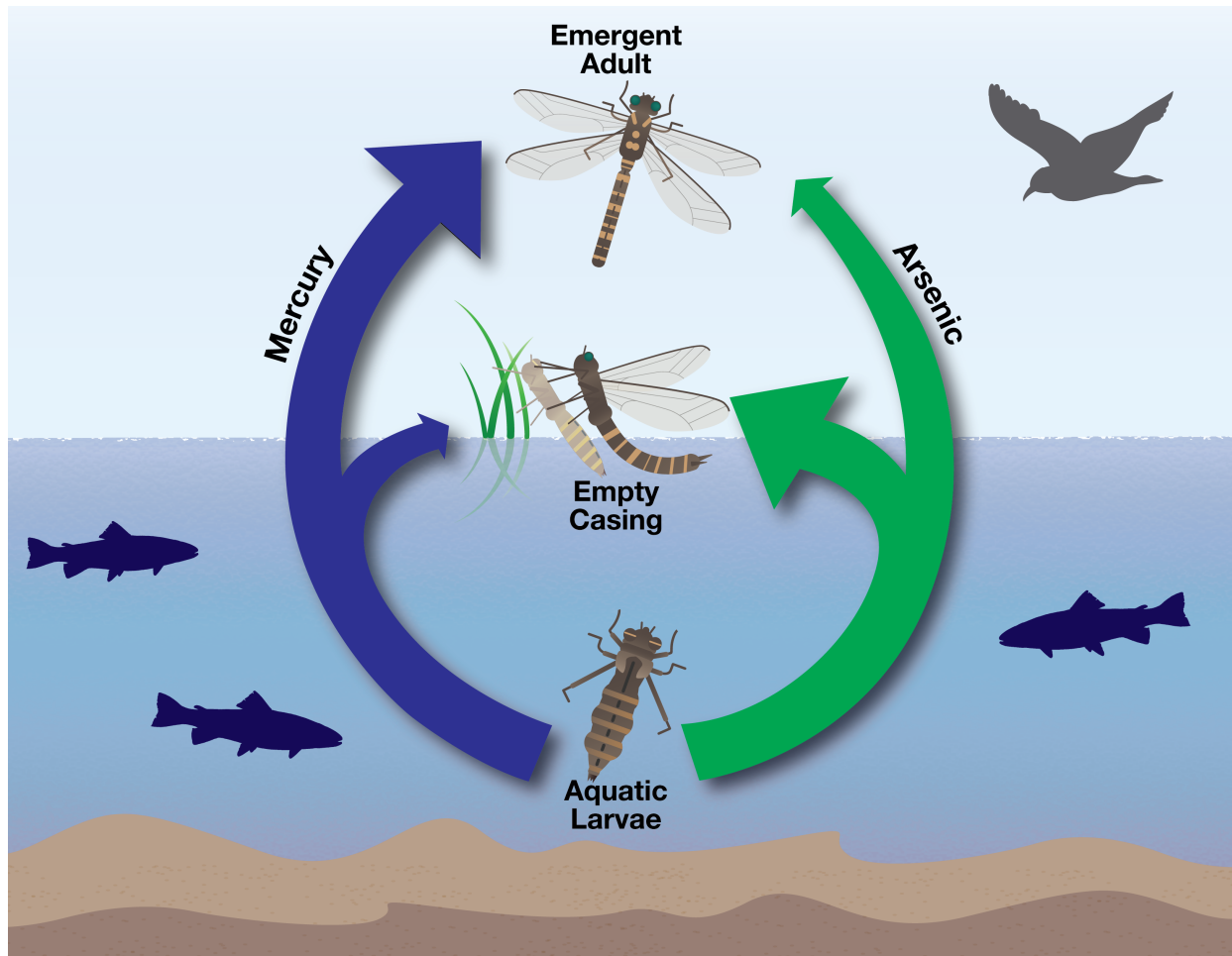


Figure 3. Conceptual figure of Hg (blue arrow) and As (green arrow) transfer via emergent insects at historical gold mine tailings sites, based on calculations of percent Hg and As lost to casings.

CHAPTER 3 SUPPLEMENTAL INFORMATION

During analysis, there was no major difference found between either genera, family, or order in terms of Hg or As accumulation, in either the larval stage or adult life stages.

However, taxonomic variation in [Hg] and [As] is provided in the following tables.

Table 3A. Order Odonata [Hg] (ppm, dry weight) by life stage from each data collection site.

Site Code	Larvae	Casings	Adults
	Mean ± SD (n)	Mean ± SD (n)	Mean ± SD (n)
MB REF	0.16 ± 0.04 (167)	0.03 ± 0.05 (6)	0.42 ± 0.32 (9)
MR REF	0.42 ± 0.19 (166)	0.21 ± 0.19 (6)	-
CB	0.71 ± 0.28 (593)	0.72 ± 1.27 (6)	0.93 ± 0.61 (15)
LC	0.97 ± 0.38 (510)	0.11 ± 0.26 (79)	1.32 ± 0.83 (37)
MP	0.99 ± 0.63 (468)	0.86 ± 0.76 (47)	1.03 ± 1.05 (26)
MR	0.77 ± 0.46 (172)	0.43 ± 0.49 (11)	-
OS	4.70 ± 2.23 (232)	8.80 ± 1.66 (3)	4.54 ± 2.78 (3)
Reference Sites Combined	0.25 ± 0.17 (333)	0.10 ± 0.15 (12)	0.42 ± 0.32 (9)
Tailings Sites Combined	1.57 ± 1.82 (1975)	0.64 ± 1.48 (146)	1.28 ± 1.16 (82)

Table 3B. Order Ephemeroptera [Hg] (ppm, dry weight) by life stage from each data collection site.

Site Code	Larvae	Casings	Adults
	Mean ± SD (n)	Mean ± SD (n)	Mean ± SD (n)
MB REF	0.15 ± 0.10 (13)		
MR REF	0.42 ± 0.38 (158)	0.06 (1)	0.80 ± 0.95 (12)
CB	0.71 ± 0.29 (125)	0.25 ± 0.13 (2)	0.61 ± 0.17 (4)
LC	1.00 ± 0.76 (357)	0.32 ± 0.56 (8)	1.20 ± 0.55 (9)
MP	4.28 ± 4.02 (3)		
MR	0.80 ± 0.41 (122)		1.17 ± 0.97 (7)
OS	-	-	-
Reference Sites Combined	0.32 ± 0.33 (171)	0.06 (1)	0.80 ± 0.95 (12)
Tailings Sites Combined	1.07 ± 1.19 (607)	0.31 ± 0.50 (10)	1.07 ± 0.70 (20)

Table 3C. Order Plecoptera [Hg] (ppm, dry weight) by life stage from each data collection site.

Site Code	Larvae	Casings	Adults
	Mean ± SD (n)	Mean ± SD (n)	Mean ± SD (n)
MB REF	-	-	-
MR REF	0.15 ± 0.13 (39)	0.15 ± 0.17 (21)	0.26 ± 0.19 (57)
CB	-	-	-

LC	-	-	-
MP	-	-	-
MR	0.64 ± 0.20 (46)	-	0.44 ± 0.21 (78)
OS		-	-
Reference Sites Combined	0.15 ± 0.13 (39)	0.15 ± 0.17 (21)	0.26 ± 0.19 (57)
Tailings Sites Combined	0.64 ± 0.20 (46)	-	0.44 ± 0.21(78)

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