Evaluation of a risk management strategy for wetland sediment contaminated with gold mine tailings using bloodworms (*Chironomus dilutus*)

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Abstract

Wetlands in Nova Scotia (NS) have been contaminated by mercury (Hg) and geogenic arsenic (As), the waste products of gold mining during the 1800s. These elements have since remained in the environment, bioaccumulating in benthic species and transferring through higher trophic levels. Here, we evaluated using a reactive amendment (R) composed of zero-valent iron (ZVI), supported by a protective capping (PC) of silica sand, ZVI, bentonite, and zeolite as a risk management strategy for impacted wetlands. We examined the treatment's ability to reduce contaminant toxicity to the freshwater larval invertebrate *Chironomus dilutus,* commonly known as bloodworms, in a laboratory experiment. Additionally, in preparation for a field mesocosm test assessing the *in situ* success of the treatment at Muddy Pond in NS, a pilot cage test was conducted to assess potential cage effects on chironomid survival and identify an appropriate cage mesh size among 200 µm, 243 µm and 300 µm that would allow chironomids with the most sediment exposure while preventing them from escaping. There was 100% survival in the control cages, indicating that the cages were not a considerable source of mortality in chironomids. The highest survival in contaminated sediment was for cages with 243 µm mesh. The toxicity test confirmed that total water Hg and As concentrations overlying the contaminated sediment were significantly reduced by at least 71% and 99% respectively, when treated with both R and PC. Chironomid survival significantly increased from 45% in the contaminated sediment to 90% when treated, while growth significantly increased by 36.5% and Hg bioaccumulation decreased by 42%. Our study indicates that this risk management strategy is safe for chironomids and can successfully reduce sediment toxicity to this invertebrate.

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1.0 Introduction

1.1 History of Gold Mining in Nova Scotia

Nova Scotia has a rich history of gold mining dating back to the mid-1800s (Bates, 1987). In 1860, John Gerrish Pulsiver, a farmer from Musquodoboit, found a quartz boulder containing gold in the Tangier River and is credited with launching the first gold rush in Nova Scotia (Bates, 1987). In the following months, many gold discoveries were made at Tangier, Goldenville, Lawrencetown, The Ovens, Wine Harbour, Waverley, Country Harbour, Isaacs Harbour and Gold River. Approximately 360 gold mines were established throughout mainland Nova Scotia from Yarmouth to Guysborough County. These mines were separated into 64 mining districts, formally demarcated by the provincial government in the late 1800s and early 1900s (Parsons et al., 2012).

There have been three major gold rush periods in Nova Scotian history, the first from 1861-1876, the second from 1882-1903, and the third from 1935-1943 (Bates, 1987). The initial discoveries of gold during the first gold rush were through the excavation of surficial quartz veins and trenching (Bates, 1987). During this period, the only method for gold extraction involved physically separating the quartz rocks from the surrounding slate and greywacke boulders using pickaxes and shovels (Bates, 1978). The quartz rocks were then primarily processed at all mines using stamp milling and mercury (Hg) amalgamation. A stamp mill consisted of a set of vertical iron or steel rods called stamps that were mechanically lifted and repeatedly dropped on the quartz rocks, crushing them into a fine silt-like mixture and exposing the gold fragments (Bates, 1987). Fresh water from nearby sources transformed this mixture into a slurry, which was then washed over copper plates coated with mercury (Parsons et al., 2012). Non-refractory gold on the milled particulates would form an amalgam with mercury (Bates, 1987), and could regularly be scraped off into an amalgam tray, transferred to a retort, and heated to a temperature where the mercury evaporated, leaving the crude gold to

be recovered and refined (Parsons et al., 2012).

To maintain satisfactory recovery rates, one ounce (oz) of mercury was generally used for each ounce of gold presumed to be within the ore (Philips, 1867). A report by Hind (1872) suggested using 1.2 oz. of mercury for amalgamation per ounce of gold. Other reports indicate that up to thrice these amounts of mercury were utilized at some mines (Kussisto, 1978). Despite the lack of experience and the use of crude techniques, an estimated 208,942.85 troy ounces (t oz) of gold were produced during the first gold rush (Bates, 1987). Although gold production increased due to expansion into new areas along the Southern and Eastern Shores, poor mining methods and substandard management led to the end of the first gold rush in the mid-1870s (Bates, 1987).

Gold extraction and processing became more effective and organized during the second gold rush (Bates, 1987). Ore production was accelerated with dynamite, allowing workers to blast larger boulders and dig deeper (Bates, 1987). In the 1880s, the introduction of cyanide leaching to the extraction process and the increased use of machinery and mills allowed for the effective processing of low-quality ore (Parsons et al., 2012). Around the 1890s, steps were taken to increase gold yields by improving the extraction processes (Parsons et al., 2012). Gravity separation, ore roasting, chlorination, and cyanidation were used with mercury amalgamation at some mines to recover gold from sulphide minerals and/or amalgamation waste material and increase the recovery of refractory gold (Parsons et al., 2012).

Since a large proportion of the gold in Nova Scotia is free-milling, individual gold particles can be freed by crushing their ores via stamp milling. However, gold is also present in sulphide minerals such as arsenopyrite and cannot be recovered by mercury amalgamation (Parsons et al., 2012). Several gravity separation devices, such as shaking tables, Frue vanners, and Wilfley tables, separated the sulphide minerals from the waste material left from the amalgamation plates based on their relatively high specific gravities (Parsons et al., 2012).

Gold was then recovered from these sulphide minerals through cyanidation, which involved leaching the gold using sulphuric acid, sodium hypochlorite, or sodium cyanide solutions (Parsons et al., 2012). Other chemicals were added during this extraction process, such as lead nitrate, which limited the transformation of cyanide to ferrocyanides and sulphocyanates, and zinc dust, which precipitated gold in the cyanide solutions (Parsons et al., 2012). Utilizing cyanidation has been disputed since the late 1800s due to the toxic effects of cyanide and other chemicals on the environment (Bates, 1987). During the second gold rush, 497,842.75 t of oz gold was produced (Bates, 1987).

The third gold rush began due to increased demand for insecticides containing arsenic (As) in the early 1920s, based on a report by the U.S. Bureau of Entomology (Hurst, 1927). Administrators at many gold mines in Nova Scotia renewed their hunt for gold and improved their recovery of arsenopyrite, a mineral associated with gold deposits. A survey in 1924 found approximately 1000 tons of arsenical concentrates accumulated at various mines around Nova Scotia (Hurst, 1924). The weathered remains of these high-arsenic concentrates have been left exposed near several old mill structures around the province (Parsons et al., 2012).

Mine waste, commonly called tailings, left behind from decades of gold mining in Nova Scotia, can contain high concentrations of mercury and cyanide that were added during the extraction phases, in addition to other potentially toxic elements like arsenic (As), antimony (Sb), lead (Pb) and copper (Cu) that are found naturally in the ore (Parsons et al., 2012). Henderson (1935) estimated that between 1862 and the mid-1940s, 10–25% of the mercury used in gold mining, amounting to 3700 to 9100 kg (Parsons et al., 2012), was lost to the environment in Nova Scotia through the dispersal of fine amalgam particles, evaporative loss during retorting, and negligent handling by mill workers.

It is widely recognized that this approximation of lost mercury may be a minimum as the actual volume of gold produced was often underreported to evade paying governmental

fees (Parsons et al., 2012). Over 3,000,000 tonnes of tailings were generated throughout the three gold rush periods (Parsons et al., 2012). Most of these tailings and processed chemicals were directly slurried into low-lying regions, including rivers (Little et al., 2015), the Shubenacadie River headwater lakes (Mudroch & Clair, 1986), swamps (Wong et al., 2002), and wetland areas surrounding mill sites (Parsons et al., 2012). This resulted in the formation of tailings flats, which can span 1 km^2 in surface area and measure several meters in depth (Drage, 2015). Over time, these tailings have migrated downstream through rivers and streams and have been found up to 2 km from the original dumping site (Drage, 2015).

Although most historical gold mining sites have been abandoned for a long time, mercury and arsenic, which do not degrade, have been found in the tailings produced from mining over a century ago (Drage, 2015). For a short period in the early 2000s, an increase in the price of gold resulted in the establishment of new gold mines, and companies began investigating the possibility of extracting gold from the tailings left at old mines (Parsons et al., 2012). In parts of Nova Scotia, in addition to artisanal and small-scale mining (ASM), large scale gold mining practices are quickly expanding (Mineral Management Division, 2024), although the amount of gold produced is considerably lower than any of the past gold rushes (Parsons et al., 2012). Additionally, the business has become more regulated, requiring upcoming mining companies to invest in scientific studies to effectively manage and mitigate possible environmental impacts (Mineral Resources Act, 2016). Unfortunately, these regulations cannot erase the legacy of historical gold mining and processing across Nova Scotia since the high concentrations of mercury and arsenic in the tailings from ASM and the lengthy duration of their existence in the environment of 500 to 1000 years, turn these deposits into contamination sources that last centuries after the closure of the mine (Martínez-Trinidad et al., 2013). Thus, the impact of the historical tailings on the environment will be a major concern for years to come (Sprague & Vermaire, 2018).

1.2 Environmental Impact of Mercury and Arsenic

Mercury is a common environmental contaminant with natural sources such as volcanic eruptions (Azevedo-Pereira et al., 2012) and anthropogenic origins through coal burning and industrial discharge (Morel et al., 1998; Wolfe et al., 1998). Because it is extremely volatile, it can remain in the atmosphere for approximately a year (Lindberg et al., 2007) and be deposited in distant regions (Morel et al., 1998). Among the toxic forms of mercury, methylmercury (MeHg) is of particular concern. Methylmercury is formed intracellularly by anaerobic microorganisms through the methylation of inorganic divalent mercury (Hg^{II}) (Kerin et al., 2006), and is thereby introduced into wetland ecosystems from gold mine tailings (Esdaile & Chalker, 2018).

Methylmercury is highly bioavailable and lipophilic, allowing it to pass through membranes, including the blood-brain barrier and placental barriers (Park & Zheng, 2012), to reach vital organs and accumulate in the brain (CCME, 2003; Doe et al., 2017; Edmonds et al., 2010). Furthermore, methylmercury accumulates faster than it is eliminated from the body, making it more likely to biomagnify through food webs, particularly in wetland ecosystems (Trudel & Rasmussen, 2006) and bioaccumulate in higher predators (O'Driscoll et al., 2005). Therefore, methylmercury concentrations generally increase with the age/size of an organism and its trophic level in the food web (Wiener & Spry, 1996). Numerous studies have found that the highest proportion of total mercury (THg) accumulation in aquatic organisms is composed of methylmercury (Lavoie et al., 2013). It is a neurotoxic compound with behavioural, neurochemical, hormonal, and reproductive effects on wildlife (Scheuhammer, 1987; Wolfe et al., 1998), negatively impacting embryo development and vertebrate nervous systems (Clark et al., 2021). Humans can be exposed to methylmercury by consuming contaminated fish and fish products (Fitzgerald & Clarkson, 1991), which causes cardiovascular and neurological issues (Salonen et al., 1995). Unfortunate industrial pollution incidents, such as that in

Minamata, Japan, highlight the extreme toxicity of methylmercury and the importance of finding solutions to prevent its bioaccumulation and biomagnification through trophic levels (Harada, 1995).

Arsenic, a metalloid, is one of the most common environmental pollutants causing worldwide problems (Du et al., 2021). It occurs naturally through volcanic eruptions and anthropogenic activities such as smelting, coal combustion, and pesticide and fertilizer application to aquatic environments (Williams et al., 2009). Arsenic can form over 300 naturally occurring compounds (Williams et al., 2009), each with varying toxicity levels to humans and other organisms (Cullen & Reimer, 1989). The toxicity of arsenic depends greatly on its concentration, speciation (oxidation state), bioavailability, and the specific detoxification mechanisms employed by organisms exposed to it (Cullen & Reimer, 1989). Generally, as opposed to mercury, inorganic arsenic species, arsenite (As^{III}) and arsenate (As^{V}), show higher toxicity than the organic forms (Ng, 2005), and are known carcinogens (Straif et al., 2009).

Arsenic enters aquatic ecosystems through land runoff, atmospheric deposition, and the direct release of wastewater (Smedley & Kinniburgh, 2002), with As^{III} and As^{V} entering freshwater systems through the erosion of mine tailings (Campbell & Nordstrom, 2014). Arsenic accumulates in organisms through direct contact or the ingestion of contaminated water, sediment, and/or organic matter (Rahman & Hasegawa, 2012). Although its biomagnification potential in the food web is reduced as compared to that of methylmercury (Sharma & Sohn, 2009), high levels of arsenic, especially arsenobetaine, have been found to bioaccumulate in aquatic organisms through trophic transfer (Maher et al., 2018; Waring & Maher, 2005). Sakata et al. (2015) reported a significant arsenic biomagnification effect in zooplankton, cephalopods, and fish in Suruga Bay, Japan. Although prolonged exposure to arsenic has been linked to skin, liver, and lung cancer in humans (Doe et al., 2017), its biomagnification potential is still undetermined. Therefore, identifying and remedying

contaminated drinking water sources and aquatic ecosystems is essential.

The recent development of land close to old mining sites has generated an increased interest in identifying the presence of tailings and evaluating their potential risks to humans and the environment (Parsons et al., 2012). A comprehensive study on sediment and surface water concentrations at 14 historical gold mining districts in Nova Scotia is provided by Parsons et al. (2012), who found that over 99% of the samples tested for arsenic concentrations exceeded the sediment and soil quality guidelines provided by The Canadian Environmental Protection Act, 1999, while mercury concentrations exceeded sediment quality guidelines in over 71% of the samples and soil quality guidelines in over 20% of the samples (Parsons et al., 2012). LeBlanc et al. (2020) provide a review of the various plants and animals in terrestrial and aquatic ecosystems near Nova Scotia's historical gold mines and their tailings deposit sites, which have been observed to show mercury bioaccumulation. Toxic inorganic arsenic species have been found to bioaccumulate in several terrestrial and aquatic species within Nova Scotia, such as mammals (Moriarty et al., 2011; Saunders et al., 2011), plants (Koch et al., 2007; Saunders et al., 2011), amphibians (Moriarty et al., 2013; Saunders et al., 2011) and invertebrates (Chapman et al., 2016; Moriarty et al., 2009). A study evaluating the distribution and bioavailability of mercury and arsenic deposited in the Shubenacadie River headwater lakes due to the gold mining operations reported that the biota, sediment, and water in Muddy Pond in Waverly had especially high concentrations of the contaminants (Metcalfe-Smith & Mudroch, 1985). Hence, to preserve the ecosystems in these areas, there is a need for risk management strategies to reduce the toxicity of the contaminants.

1.3 Risk Management of Contaminated Wetlands

The risk management approach to be used at a specific contaminated site should ideally take into consideration the bioavailability and chemistry of the contaminants present. However, in most cases, excavating the contaminated material to dump, treat, or bury elsewhere is the

traditional risk management solution. The major disadvantage of this method is that it is very conservative and expensive. In addition, large scale soil excavations are disruptive to native ecosystems and come with a risk of increasing contaminant mobility during the process (National Research Council, 1999). Excavating wetland areas contaminated with gold mine tailings introduces oxygen to these reduced sediments, possibly resulting in the increased mobility of mercury and arsenic from the sediments into the surface water (DeSisto et al., 2017). De Freitas et al. (2019) found that after being subjected to resuspension tests, metals showed higher concentrations in their bioavailable phases, indicating an elevation in their potential bioavailability. Hence, dredging activities could lead to an increased risk of exposure and bioaccumulation for local biota. Alternatively, risk management strategies for sediment contaminated with metals/metalloids may involve amendments, capping, phytoremediation and thermal treatments focused on decreasing the mobility *in situ*, which can limit how far a contaminant can disperse, and the toxicity of those contaminants (Peng et al., 2018). *In situ* remediation techniques that alter the chemistry of the contaminant within the soil to reduce their solubility, mobility, and bioavailability are becoming more common, especially for areas extensively contaminated with metals/metalloids such as mining sites, although typical methods such as thermal treatments, leaching processes, phytoremediation and electrolysis, are consistently found to be expensive, labour intensive and time-consuming (Biester & Zimmer, 1998; Martin & Ruby, 2003; Rowe & Hosney, 2013). Additionally, most risk management strategies are still being researched and are currently not accepted by regulatory agencies, unlike the traditional methods of excavation and capping.

The objective of capping is to decrease the mobility, solubility, and transfer rate of the contaminant within the sediment by either stabilizing the sediment or physically/chemically isolating the contaminant (Mohan et al., 2000). Traditional isolation capping is a non-intrusive and cost-effective method for remediating contaminated sediments using sandy materials like

clean sediment, sand, gravel or clay applied in thick layers to physically contain contaminants and consequently reduce exposure (Peng et al., 2018). Reactive amendments are a form of capping that aim to decrease the mobility, toxicity, and bioavailability of contaminants, using chemical reactions between their components and the contaminants (Peng et al., 2009). Multicomponent capping includes reactive amendments such as rock phosphate, lime, or zeolite, either mixed or layered in specific proportions with sandy materials, and then placed on top of the contaminated sediment, providing physical containment and treatment simultaneously (Peng et al. 2018). Additionally, less material is needed for multicomponent capping as compared to traditional isolation capping. Amendments that have high cation exchange capacities can lower metal mobility and solubility, consequently reducing bioavailability in the sediment (Peng et al., 2009). Generally, they are environmentally friendly and economically reasonable (Peng et al., 2009).

There are a variety of reactive materials available for stabilising mercury, such as biochars (Ahmad et al., 2014; Li et al., 2017), activated carbon (Gilmour et al., 2013; Patmont et al., 2015), zero-valent iron (Weisener et al., 2005), and sulfur and iron mixtures (Zhong et al., 2018). However, many materials needed to produce reactive amendments are expensive and impractical for large contaminated sites. Another issue is that reactive amendments are specific to a particular contaminant and therefore, while some amendments can treat mercury contamination, they may not be appropriate for arsenic and vice versa. Zero-valent iron (ZVI) is an inexpensive material that has long been used in permeable reactive barriers for the treatment of contaminated ground water (Lewis et al., 2016). The high cation exchange capacity and reductive properties of ZVI have been shown to reduce arsenic toxicity while its adsorptive properties have been credited with reducing dissolved mercury concentrations in synthetic aerobic and anaerobic systems (Lewis et al., 2016). Furthermore, nanoscale zero-valent iron (nZVI), an engineered nanomaterial, is an effective reducing agents for metals,

metalloids, pesticides, pharmaceutical compounds, and inorganic and organic contaminants (Arshadi et al., 2017; Gil-Díaz et al., 2017). Besides being relatively low-cost and having low toxicity (Arshadi et al., 2017; Gil-Díaz et al., 2017), recent research has shown that nZVI is capable of immobilizing arsenic and mercury in brownfield soil (Gil-Díaz et al. 2017) and water (Arshadi et al. 2017). Lewis et al. (2016) investigated the ability of nZVI to reduce methylmercury production and bioavailability to benthic organisms in wetlands, using a field and laboratory test. They reported that methylmercury concentrations in sediment treated with nZVI were lower than in untreated sediment, and *Lymnaea stagnialis,* (great pond snail), accumulated less methylmercury in nZVI treated sediment than in untreated sediment (Lewis et al., 2016).

In wetland areas, due to the movement of water over the sediment, any amendment added would likely be eroded quickly. Therefore, it is essential to use a protective capping, a layer placed on top of the amendment with the objective of preventing its erosion and improving its remediation capabilities (Peng et al., 2018). Bentonite, which is mostly used as a binding agent and adsorbent, has been found to reduce the erosion of the top layer of sediment caused by water (Gailani et al., 2001). Therefore, bentonite which also increases the soil-water content, is an ideal component for an *in situ* treatment for wetland remediation. Zeolite allows dissolved solids, gases, or liquids to adhere to its surface and is thus commonly used as a commercial adsorbent. It improves the binding efficacy of nZVI to mercury and has been used as a component of the protective capping in a number of studies involving mercury remediation (Zhang et al. 2009). While selecting a treatment for a wetland area, it is crucial to ensure that it is not toxic to sensitive organisms in the ecosystem.

1.4 Toxicity Testing using *Chironomus dilutus*

Benthic, sediment-dwelling, organisms play a vital role in the food web by forming the link to higher trophic levels in aquatic environments (Soon-Mi et al., 2006). Contamination

affecting these aquatic ecosystems may affect the distribution and abundance of benthic fauna, and any adverse effects of pollutants on these organisms may consequently be reflected in the whole ecosystem (Fleeger et al., 2003). Generally, the potential toxicity of contaminated aquatic sediments is evaluated using benthic macroinvertebrates to conduct laboratory experiments (Benoit et al., 1997). Among the aquatic invertebrates, the larvae of nonbiting midges (Chironomidae, Diptera) are sensitive to toxic substances, ubiquitous, and distributed globally (Hall et al., 1970). The morphological characteristics of chironomid larvae are diverse with some species displaying a bright red body colour due to which they are commonly called bloodworms. This coloration is a result of a type of hemoglobin that fixes oxygen and may metabolize environmental contaminants (Osmulski & Leyko, 1986). The combination of this ability and their symbiotic relationship with endogenous bacterial communities allows them to tolerate and thrive in polluted environments (Senderovich & Halpern, 2013; Weber & Vinogradov, 2001). Not only are chironomids the most abundant group of insects inhabiting freshwater bodies, they also have an important role in the food chain as a major source of food for many fish species among other vertebrates and local invertebrates (McLachlan et al., 1995). Chironomids generally constitute a significant proportion of the benthic biomass and are vital in cycling detritus into and from sediments via burrowing, ingestion, and defecation (Adams et al., 2008). Although exhibiting a level of tolerance, chironomids may display morphological modifications such as mouth deformities and undergo molecular and behavioural changes due to chronic exposure to contaminants (Azevedo-Pereira & Soares, 2010).

Chironomus dilutus, formerly known as *C. tentans* (Butler et al., 1999), has been used extensively in short-term evaluations of the acute and sublethal toxicity of sediments and water contaminated with metals (Giesy et al., 1988; Nebeker et al., 1984; Wentsel et al., 1977; West et al., 1994), and standard methods have been developed for this invertebrate using 10-day exposure tests (ASTM, 1995; EPA, 1994; Environment Canada, 1997; Ingersoll et al., 1995).

Besides producing a large number of progeny in a short period of time (EPA, 1994), *C. dilutus* is a good model species for chronic toxicity testing because it normally completes its life cycle in a relatively short period of time (25-30 days at 23°C) (EPA, 2000) and is relatively easy to culture and handle in the lab (EPA, 1994). The larvae remain in close contact with the sediment throughout their development, living in self-constructed casings and filter feeding or ingesting sediment present within the upper few centimetres of lakes and rivers (Adams et al., 2008). In a number of studies, results from short-term exposure tests using *C. dilutus* have been shown to be predictive of population-level effects (Giesy et al., 1988; Sibley et al., 1997). Tests using *C. dilutus* have commonly started with second- or third-instar larvae (10-14 days old) and continued for 10-14 days after which the sediment is sieved and larvae are recovered (Liber et al., 1996) to examine a variety of developmental endpoints such as growth, measured by dry weight and/or head capsule width, and survival (EPA, 2000). Liber et al. (1996) reported that although some contaminated sediments do not cause mortalities to *C. dilutus*, they may significantly inhibit larval growth.

1.5 Research Goals

This research project assesses the effectiveness of a treatment for wetland sediment contaminated with legacy gold mine tailings by examining survival, growth and mercury bioaccumulation in *Chironomus dilutus* larvae. The treatment consists of a reactive amendment of zero-valent iron and a protective capping, containing sand, zeolite and bentonite. The goal is to determine whether such a treatment is safe for *C. dilutus* at contaminated wetlands and if it can be used to reduce the toxicity of the tailings and bioavailability of mercury. This project, as part of the legacy gold mine contaminants research led by the Dynamic Environment and Ecosystems Health Research (DEEHR) team at Saint Mary's University, will enhance our understanding of the impact of historical human activity on aquatic ecosystems and the use of *in situ* treatments to remediate wetlands.

2.0 Materials and Methods

2.1. *Chironomus dilutus* Rearing Protocols

Chironomus dilutus (formerly known as *C. tentans*, ITIS Taxonomic Serial No. 129325) egg sacs were obtained from Aquatic Research Organisms, Inc. (Hampton, NH, USA) and acclimated to laboratory conditions following standard recommended practices (Canada Environmental Protection Series, 1997). Chironomid larvae cultured from the egg sacs were housed in a 52 L polyethylene lidless container (culture chamber) measuring 50.8 cm x 30.5 cm x 35.6 cm with a 1 mm mesh screening covering the open surface. To allow the larvae to develop, the culture chamber contained a 3 cm layer of silica sand (#00; Atlantic Silica, Poodiac, NB, Canada) rinsed with water purified through reverse osmosis (RO), and was filled with 20 L of aerated culture water with a pH of 7.2-8.2 prepared using the method provided by the Canada Environmental Protection Series (1997). Water quality (temperature, dissolved oxygen (DO%), pH, salinity, specific conductivity (SPC), total dissolved solids (TDS), oxidation-reduction potential (ORP), ammonia, and alkalinity) was measured weekly and maintained through water changes and gentle aeration using an air pump, standard airline tubing, and glass pipettes. Chironomid larvae were exposed to 16 h of fluorescent light daily (0800–2400) at 500-1000 lux, and water temperatures were held at 21-25 °C using aquarium heaters. These conditions were maintained consistently for every laboratory experiment. The larvae were fed a slurry of finely ground commercial fish flakes (TetraFin Goldfish Flakes®, Blacksburg, VA, USA) and RO water made of 4.0 mg of dry solids/mL once a week.

2.2 Reference Toxicity Test

To determine the relative sensitivity of the laboratory culture of *C. dilutus* larvae under standardized test conditions and assess the precision and reliability of the data produced from the subsequent sediment toxicity test, a 96-hour reference toxicity test was conducted in July 2023 using second instar larvae and chemical grade sodium chloride (NaCl). Commonly used

as a reference toxicant (Environment Canada, 1997), sodium chloride shows higher *C. dilutus* mortality at higher concentrations. Deviations from this trend suggest that the specific laboratory culture may be atypically sensitive and thus not appropriate for future toxicity testing. As recommended by the Canada Environmental Protection Series (1997), culture water was used as a control, while five test solutions were prepared using NaCl and culture water at concentrations of 10.0 g/L, 3.2 g/L, 1.00 g/L, 0.32 g/L, and 0.10 g/L. A metal spatula was used to evenly spread 25 mL of rinsed silica sand (#00, Atlantic Silica) around the base of 300 mL beakers. With minimal disturbance to the sediment layer, 200 mL of either a test or control solution was gently poured into each prepared beaker and left for 24 hours to settle.

Using transfer pipettes, 10 second instar larvae were randomly transferred into each beaker and fed 0.6 mL of the fish flake slurry. The beakers were then covered with finely perforated parafilm to prevent the entry of physical contaminants but still allow for natural air exchange. The test was conducted with no replicates. Daily observations were made for dead and lifeless larvae in each beaker. After four days, chironomids were gently removed from individual beakers using transfer pipets and placed into clean Petri dishes. To determine whether motionless individuals were dead, they were gently prodded with a fine-tipped needle.

2.3 Acute Sediment Toxicity Test

A 10-day laboratory acute sediment toxicity test was conducted in August 2023 to examine survival, growth and mercury bioaccumulation in *C. dilutus* larvae exposed to treated and untreated wetland sediment contaminated with legacy gold mine tailings. The test aimed to determine whether the treatment was safe for *C. dilutus* at contaminated wetlands and if it could be used to reduce the toxicity of the tailings and the bioavailability of mercury.

2.3.1 Sediment Collection and Preparation

Sediment containing tailings was collected from a wetland site at Muddy Pond (MP) (44°47'13.8"N 63°36'32.3"W) located within the Waverley gold mining district in the town of Waverley, Nova Scotia (Fig. 1). The precise sampling location was selected to represent the most contaminant-rich sediment area of the wetland based on a province-wide survey of multiple tailing-impacted wetlands conducted by LeBlanc et al. (2019). The silverish-grey tailing layer immediately below the more recent thin organic sediment layer at the top was collected using a shovel and inserted into several Ziploc® bags, tightly sealed to minimize contact with air. The bags were placed inside a cooler with ice packs for transportation before being refrigerated at 4 °C and stored for approximately two weeks until the start of the test. Moisture content for this sample of Muddy Pond sediment was calculated (Eqn. 1) using

Figure 1. Map of the Canadian province of Nova Scotia, located on the eastern seaboard of North America. The inset map shows the location of the research sites. The site contaminated with legacy gold mine tailings is located at Muddy Pond (MP) in the Waverley gold mining district. The reference site is at Second Lake (SL) in Lower Sackville.

aluminium weighing boats to measure the weight of 3-5 g of sediment before and after being dried in a gravity convection oven for 24 hours at 90-105℃.

$$\% \text{ moisture} = \frac{\text{sediment wet weight (g)} - \text{sediment dry weight (g)}}{\text{sediment wet weight (g)}} \text{ x } 100 \quad (1)$$

Second Lake (SL) (44°46'50.1"N 63°39'42.8"W) in Lower Sackville, Nova Scotia (Fig. 1) has sediment with similar properties to Muddy Pond but has not been impacted by legacy gold mine tailings and was collected and weighed as a reference sediment for this test in the same way as sediment from Muddy Pond. As a laboratory control, artificial sediment (AR) was prepared to be similar to tailing material in particle size distributions, organic carbon content, buffering capacity, and pH. Artificial sediment has been used successfully in pilot acute sediment toxicity tests (Chapman et al., 2019) and was produced by first creating a mixture composed of a dry weight of 18% silica sand (#00, Atlantic Silica), 72% silica sand (400 mesh, The Pottery Supply House, Oakville, ON, Canada), 5% kaolin clay (The Pottery Supply House), and 5% sphagnum peat moss (ASB Greenworld, Stuttgart, Germany). To reduce the acidity caused by the peat moss, calcium carbonate ($CaCO_3$) was added at 0.57% of sediment dry weight to the mixture until the pH was 6-7. RO water was added until a moisture content similar to the collected wetland sediment was reached (approximately 25%).

2.3.2 Protective Capping Preparation

The protective capping (PC) was prepared two weeks before the start of the test. A mixture composed by dry weight of 87.5% silica sand (#00; Atlantic Silica), 2.5% bentonite (325 mesh; The Pottery Supply House), and 10% zeolite (300 mesh; Progressive Planet Products, Kamloops, BC, Canada) was prepared. Based on the dry weight of this mixture, five percent of zero-valent iron (cc1200; Connelly-GPM, Inc., Chicago, IL, USA) was added along with RO water at a ratio of 4 g of dry mixture to 1 g of water. The resulting material was molded into a thin ‘puck’-shaped disc and left to air dry.

2.3.3. Experimental Setup

Eight days prior to the start of the test, twelve sub-samples of the contaminated sediment, and four sub-samples of both, the reference and control sediments, each weighing 265.7 g were inserted into 1 L glass jars (test chambers) to form a 3 cm layer in each test chamber (Fig. 2). This thickness of the sediment layer is important as possible real-world applications will consider the depth of wetland tailings when measuring how much treatment is to be added. A 10 g sediment sample was collected from each test chamber in centrifuge tubes and refrigerated at 4 °C. Each test chamber received 600 mL of culture water poured gently along the side to minimize disturbance of sediments. Based on the calculated sediment moisture content, eight of the test chambers containing contaminated sediment were treated with the reactive amendment zero-valent iron (cc1200; Connelly-GPM, Inc) at 37.5 g/kg dry weight of sediment, evenly spread across the surface of the sediment, and left undisturbed for 24 hours (Fig. 2). After the ZVI had completely settled, a single PC 'puck' was gently released into the overlying water of four test chambers containing the contaminated sediment and ZVI layer and evenly distributed using a metal spatula to form a 0.25 cm uniform layer. The suspended sediment particles were then allowed to settle for 48 hours. Figure 2 provides a summary of the experimental design used for the acute sediment toxicity test.

From the *C. dilutus* culture, the head capsule width of 20 randomly selected larvae was measured under a dissecting microscope to ensure the population was in its third instar. To reduce the chance of stress caused due to handling affecting the results of the toxicity test, these particular larvae were not used during the experiment. However, this initial measurement was considered to represent the mean head capsule width for the population. Groups of 10 larvae from the culture were randomly selected and transferred to each test chamber using transfer pipettes. The test chambers were sealed with a lid to prevent the entry of contaminants and held at 21-25 °C using a water bath. Continuous (2-3 bubbles/second) water aeration was provided

for the larvae without disturbing the sediment via an air pump, standard airline tubing, and a glass pipette placed through a hole drilled into the center of the lid. Chironomids were fed 2 mL of the fish flake slurry at the start and middle of the test.

When the test ended after ten days, 35 mL and 70 mL of the overlying water from each test chamber were collected for arsenic and methylmercury analysis, respectively. The remaining water in the test chambers was gently swirled to suspend the upper ~1 cm of sediment and slowly sieved through a 300 μm mesh sieve. The contents retrieved on the sieve were examined for chironomids. The sediment remaining in the chamber was then gently sieved through a 425 μm mesh sieve and inspected for chironomids. The head capsule width for every surviving larva was measured to the nearest 0.005 mm using an ocular micrometer. All living chironomids recovered from a single test chamber were placed in an aluminum weighing boat and rinsed with culture water to remove any external sediment. Post rinsing, the larvae were moved to clean, aluminum weighing boats with culture water and left undisturbed for 24 hours to allow for gut purging. The larvae were then transferred to clean aluminum weighing boats and placed in a gravity convection oven for 24 hours at 40 ℃, then left to cool

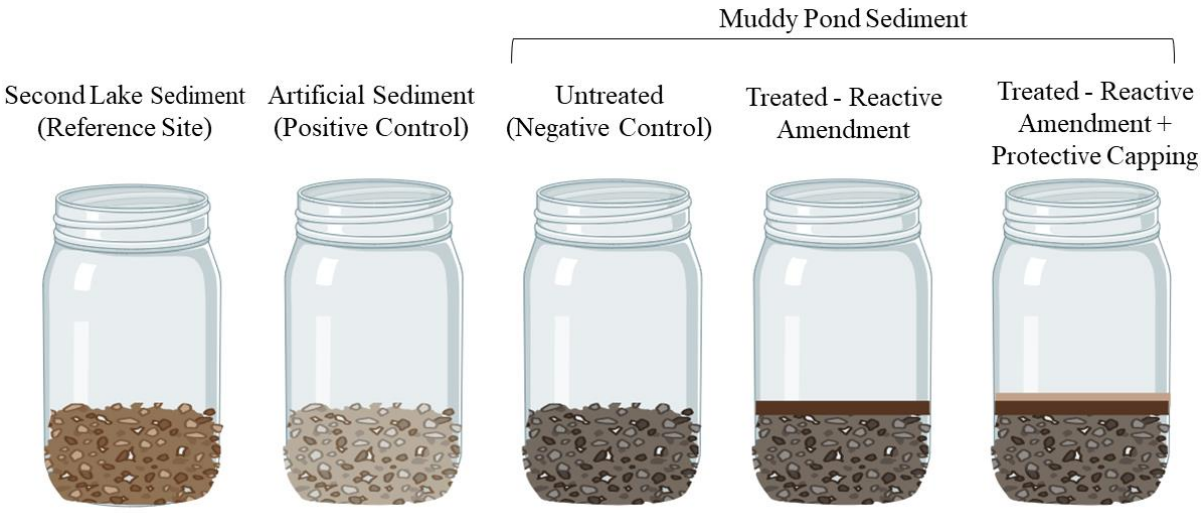

Figure 2. Schematic diagram of the experimental setup used for the acute sediment toxicity test conducted using third instar *Chironomus dilutus* larvae to examine differences in growth, survival, and mercury bioaccumulation. Each treatment had group had four replicates and ten larvae were placed in each test chamber

in a desiccator cabinet. The weight of the chironomids from each test chamber was determined using a semi-micro balance.

The dried chironomids were analysed with a DMA Direct Mercury Analyser-80.3 (Milestone Srl, Sorisole, BG, Italy) for total mercury content (THg) using nickel boats in a HEPA-filtered cleanroom laboratory. Chironomids from all replicates in a treatment group were analysed together due to the low sample mass. To minimize the risk of cross-contamination, trace-element protocols were followed, and each run started with multiple blanks, followed by a series of mercury standards (0, 5, 10, and 20 ppm), along with two certified reference materials (CRM), DORM-4 (dogfish muscle tissue) and Tort-3 (lobster hepatopancreas) from the National Research Council of Canada. For quality assurance, duplicates were run every ten samples, and to prevent contamination via carry-over of samples, two to four blanks were run between samples from different treatments.

2.4 Cage study

Results obtained from the acute sediment toxicity indicate the impact of the treatments on the chironomids under controlled conditions in the laboratory. To fully assess how the larvae would respond to the treatment at contaminated wetlands, chironomids must also be exposed to the sediment in the field. However, since they are sediment-dwelling, it is not possible to place them in the field without the risk of losing them over the test period. Therefore, it was necessary to create an enclosure that not only prevented them from escaping, but also allowed for the most sediment exposure. To examine bioaccumulation at the end of the test, larvae must be alive to allow for proper rinsing and gut purging procedures. Thus, an *in situ* caging study was conducted in October 2023 to determine which mini-cage design would allow for the most exposure to contaminated sediment and the highest survival. This study aimed to inform a field mesocosm study to be completed in the summer of 2024.

2.4.1 Cage Design

Each mini-cage was constructed from translucent 30 mL round wide-mouth polypropylene jars. Holes measuring ~2.5 cm were drilled into the sides of the jars in a zigzag pattern so that there were two rows of six alternating holes ~2-3 cm from each other, and ~5.7 cm holes were drilled into the lid and the bottom of the jars (Fig. 3). The mesh size had to be small enough to prevent third instar larvae with head capsule widths ~330-450 µm from escaping, but not so small as to result in low DO% within the mini-cages. Accounting for these factors, the holes were covered with either 200 µm, 243 µm or 300 µm Nitex mesh using silicone aquarium sealant (Marineland®, Blacksburg, VA, USA) (Fig. 3). Mini-cages were submerged in deionized water for 24 hours to remove toxic remnants from the sealant.

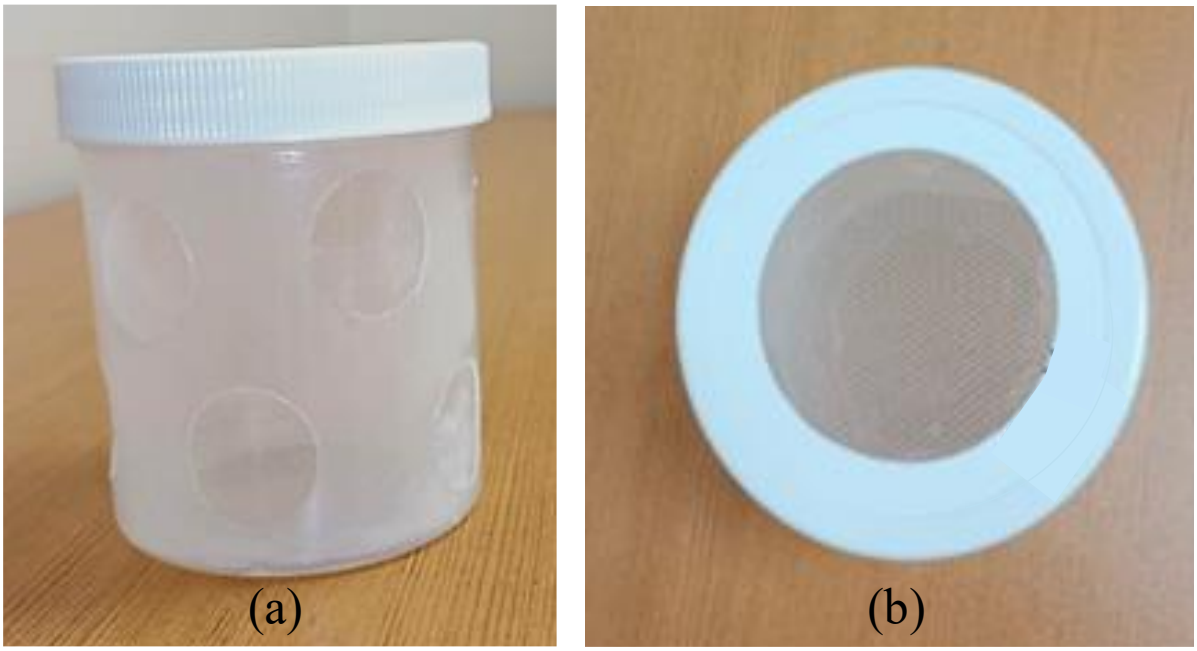

Figure 3. (a) A profile view of the mini-cage used to contain chironomid larvae in the laboratory and field survival tests. Holes are covered completely by 200 µm, 243 µm, or 300 µm Nitex mesh. (b) A top-down view of the mini-cage with larger holes in the bottom and lid to maximize exposure to sediment and allow water exchange, respectively.

2.4.2 Cage Survival Test

A 5-day caged test was conducted using clean silica sand as a substrate to determine whether the larvae could survive being enclosed in the mini-cages. Using RO water, silica sand (#00; Atlantic Silica) was rinsed thoroughly and added to two clean 5.5 gal aquariums to form

a 2 cm layer of sediment. Sediment samples were collected in PETG bottles and refrigerated at 4 °C. In each aquarium, 9 L of culture water was carefully poured down the side to ensure minimal sediment disturbance and was left undisturbed for 24 hours. Three replicate mini-cages of each mesh size (200 μm, 243 μm, 300 μm) were randomly divided between the two aquariums and inserted into the sediment without their lids using a 'screwing' motion back and forth.

Groups of 20 third instar chironomid larvae were randomly placed in each mini-cage using transfer pipettes, and the lids were secured. Over the 5-day test period, 15% water changes were conducted daily, and larvae were monitored for growth and survival. They were not fed but allowed to consume any available sediment *ad libitum*. At the end of the experiment, all live and dead chironomids within the cages were pipetted. From the overlying water in each test chamber, 35 mL and 70 mL samples were collected for arsenic and methylmercury analysis, respectively.

2.4.3 Mini-Cage Lab Test

A 5-day test of a planned field experiment was conducted *ex situ* at Saint Mary's University using water and sediment from Muddy Pond to simulate field conditions.

The overlying water from Muddy Pond was collected in 20 L polypropylene carboys and transported back to Saint Mary's University. A day prior to the start of the experiment, third instar chironomid larvae were acclimated to this water using the method described in the Canada Environmental Protection Series (1997). The procedure followed during the cage survival test in section 2.4.2 was then replicated using water and sediment from Muddy Pond instead of culture water and silica sand, respectively.

2.4.4 Mini-Cage Field Test

A 5-day *in situ* caged test was conducted at Muddy Pond in October 2023 to determine which mini-cage design allowed for the most exposure to the sediment contaminated with legacy gold mine tailings and the highest survival at the end of the test.

Two days prior to the start of the experiment, third instar chironomid larvae were acclimated to water collected from Muddy Pond. Ten hours before installing the mini-cages at Muddy Pond, third instar chironomid larvae were individually moved into 1.8 mL polyethylene cryovials using transfer pipettes. Each cryovial contained ~0.2 cm of silica sand and ~1 mL of culture water with no headspace. Cryovials were then placed compactly in Ziploc® bags, which were placed in a polystyrene box with sufficient foam padding to resist movement during transport to Muddy Pond.

Four mini-cage replicates of each mesh size were fastened to powder-coated steel ground anchors using heavy-duty nylon rope and inserted ~1-2 cm into the sediment in Muddy Pond so that they were fully submerged in the water. In centrifuge tubes, sediment samples were collected from each location where a mini-cage was installed, and placed inside a cooler with ice packs for transportation back to the University. Three water samples from the general area of the mini-cages were collected in Teflon bottles for methylmercury and arsenic analysis. Twenty chironomids were released into each mini-cage, and the lids were secured. After five days, the mini-cages were carefully removed, and the number of dead and living larvae was recorded.

2.5 Water and Sediment Analysis

To ensure acceptable conditions for the chironomids during every test, the temperature, DO%, ammonia, pH, alkalinity, and conductivity were measured regularly using a YSI multiparameter sonde and an HI 83300 photometer (Hanna Instruments, RI, USA).

The overlying water sampled during the tests for arsenic and methylmercury analysis was collected using 50 ml Luer lock syringes, filtered through 0.45 μm Teflon DigiFILTERs, and stored in digiTUBEs and PETG bottles, respectively. Arsenic samples were preserved with 1% by volume ultrapure nitric acid and refrigerated at 4 °C before being sent to the Analytical Services Unit, Queen's University (Ontario, Canada) for ICP-MS analysis. Methylmercury samples were preserved with 0.2% by volume ultrapure hydrochloric acid and frozen at -20 °C prior to being sent to Flett Research Ltd. (Winnipeg, Canada), for analysis via distillation, aqueous ethylation, purge and trap, and cold vapour atomic fluorescence spectroscopy (CVAFS) using a Tekran 2700 Mercury Analyser (Version 3; TN, USA).

Sediment samples were analysed for ultra-low determination of arsenic, mercury, iron, and other metals using inductively coupled plasma mass spectrometry (ICP-MS) analysis after modified aqua regia digestion (1:1:1 HNO_3/HCl/H_2O) by Bureau Veritas Environmental Laboratories and Specialty Services in Bedford, NS, Canada. Sample blanks were included through all stages of preparation and analysis. Duplicates were used to ensure analytical precision. A reagent blank was used to calibrate the background values and two Certified Reference Materials (OREAS45EA and DS11) were included to monitor accuracy.

2.6 Data Analysis

The differences in the test parameters for *C. dilutus* larvae, overlying water and sediment between treatments for the acute sediment toxicity test and cage studies were assessed using a parametric one-way ANOVA with post-hoc Tukey HSD tests for comparison of means. Significance was determined at $\alpha = 0.05$, $p < 0.05$, except when normality or equal variance tests failed, in which case the non-parametric Kruskal-Wallis one-way analysis of variance on ranks was used with pairwise multiple comparison Dunn's test (Holm method), and significance was determined at $p < 0.05$. Analyses were conducted using the FSA (Ogle et al.,

2023) and car (Fox & Weisberg, 2019) packages and visualized using the ggplot2 (Wickham, 2016) package in R (ver. 4.3.2; R Core Team 2023).

3.0 Results

3.1 Reference Toxicity Test

At the end of the 96-hour reference toxicity test, second instar larvae (n=10) exposed to silica sand with higher concentrations of NaCl in culture water generally showed lower survival rates (Table 1). The control containing only culture water had 100% survival and the highest NaCl concentration tested (10 g/L) showed the lowest survival (10%).

Table 1. Percent survival at the end of the reference toxicity test for *C. dilutus* larvae in silica sand exposed to different concentrations of NaCl in the culture water

Sediment	NaCl concentration (g/L)	Chironomid survival % (n=10)
Silica sand	0	100
	0.1	90
	0.32	80
	1.0	80
	3.2	60
	10.0	30

3.2 Acute Sediment Toxicity Test

3.2.1 Sediment Analyses

The concentrations of total mercury and arsenic measured from sediment samples collected before the addition of any treatments at the start of the test (day 1) varied between sediment types, and replicates within treatments. Table 2 provides the statistical method used to determine significance and the differences between treatments. Total mercury concentrations were measured below the detection limit (< 2.0 mg/kg) for both the control artificial sediment (AR) and Second Lake (SL) reference sediments and were significantly lower than the contaminated sediments (Table 2). The highest total mercury concentration measured in the contaminated sediment from Muddy Pond in the reactive amendment group (MR) at 12.5 ±

1.29 mg/kg (n=4), was significantly higher than both the sediment in the reactive amendment and protective capping (MC) group at 10.9 ± 1.56 mg/kg (n=4), and the untreated (MP) group at 10.8 ± 0.96 mg/kg (n=4) (Table 2). There was no significant difference between the total mercury concentrations in MP and MC. Concentrations of mercury in all contaminated sediments exceeded the Canadian Ministers of the Environment (CCME) Interim Sediment Quality Guidelines (ISQG) of 0.17 mg/kg and probable effect levels (PEL) of 0.486 mg/kg.

The lowest concentration of arsenic was below the detection limit (< 2.0 mg/kg) in the replicates of the control AR while SL sediment contained 5.7 ± 2.54 mg/kg (n=4). For the contaminated sediment, there was no significant difference between the arsenic concentrations in untreated MP at 20250 ± 3500 mg/kg (n=4), MR at 23750 ± 4500 mg/kg (n=4), and MC at 19750 ± 3948 mg/kg (n=4). The control and reference sediments both had significantly lower arsenic concentrations compared to the contaminated sediments (Table 2). Arsenic concentrations in all contaminated sediments far exceeded both the CCME ISQG of 5.9 mg/kg and PEL of 17 mg/kg.

3.2.2 Water Analyses

Concentrations of dissolved methylmercury and arsenic in the overlying water were measured at the end of the test (day 10) to determine if the reactive amendment and protective capping treatments reduced contaminant concentrations in the water column and therefore their mobility through the sediment. The average dissolved methylmercury concentration in the water overlying the untreated sediment MP (0.058 ± 0.013 ng/L; n=4) was significantly higher than in all other groups (Table 2). However, the concentration of methylmercury in the water overlying the sediment in MC (0.013 ± 0.003 ng/L; n=4) was significantly lower than both the control AR (0.026 ± 0.005 ng/L; n=4) and MR (0.028 ± 0.002 ng/L; n=4) (Table 2). Furthermore, there was no significant difference in the methylmercury concentrations in the water overlying the sediment in the control and MR, and the reference sediment (0.013 ± 0.001

ng/L; n=4) and MC. None of the methylmercury concentrations in any of the treatments were above the CCME guideline value for the protection of aquatic life of 4 ng/L.

As expected, the reference sediment treatments had the lowest arsenic concentrations in the overlying water column with 1.2 µg/L in one replicate and the others (n=3) measured below the detection limit (<1.0 µg/L). The highest concentration of arsenic was measured in the overlying water of the untreated sediment at 997.5 ± 230.41 µg/L (n=4) and was significantly higher than all other groups (Table 2), exceeding the CCME guideline for protection of aquatic life of 5 µg/L as well as the US EPA freshwater chronic aquatic life guideline for dissolved arsenic (150 µg/L) (US EPA, 2023). There was no significant difference in the dissolved arsenic concentration in the overlying water in the control group at 1.75 ± 0.52 µg/L (n=4) and the treated contaminated sediment groups MR and MC at 2.1 ± 1.38 µg/L (n=4) and 3.9 ± 2.25 µg/L (n=4), respectively. Both contaminated sediment groups that were treated had dissolved arsenic concentrations within the CCME limit.

General water parameters (temperature, pH, dissolved oxygen (DO%), ammonia, and alkalinity) were measured in a single replicate representative of the treatment at the beginning and at the end of the test. Temperature remained relatively constant throughout the test fluctuating between 22 °C and 24 °C. pH was near neutral to slightly basic in all treatments during the test, initially ranging from 7.65 in AR to 8.47 in the SL treatment. For most sediment types, water pH increased by ~0.5-1 unit between the initial (day 1) and final (day 10) measurements, except in the reference sediment SL where the pH decreased to 8.05. At the beginning of the test, most treatments had dissolved oxygen levels >100%, which dropped to 80-90% by the end of the test. Initial ammonia concentrations in MP and SL treatments were greater than the limit for chironomids (0.2 mg/L) but these levels fell within the limit by the end of the test. Alkalinity in all treatments, except SL, increased over the test period from ~45 mg/L to ~65 mg/L. Alkalinity concentrations in SL decreased from 148 mg/L to 47 mg/L.

3.2.3 Survival, Growth and Bioaccumulation

At the end of the 10-day acute sediment toxicity test, each test chamber was examined for chironomid survival. Each replicate of the control and reference sediments showed high survival (87.5 ± 12.6%; n=4 and 95.0 ± 10%; n=4 respectively) well exceeding the test validity criteria of a minimum average 70% survival in controls (Environment Canada, 1997). As expected, analysis of variance showed that there was significantly lower survival (55 ± 23.8%; n=4) in the untreated Muddy Pond sediment, compared to the control and reference groups (Table 2). Contaminated sediment in treatment groups MR and MC showed significantly improved survival at 82.5 ± 12.6% (n=4) and 90 ± 8.2% (n=4) respectively, compared to untreated sediment in MP (Table 2). There was no significant difference in survival between the control, reference and treated contaminated sediment groups (Fig. 4). Figures 5a and 5b demonstrate the relationship between the concentration of total mercury and arsenic respectively in the sediment at the start of the test before the addition of any treatments and

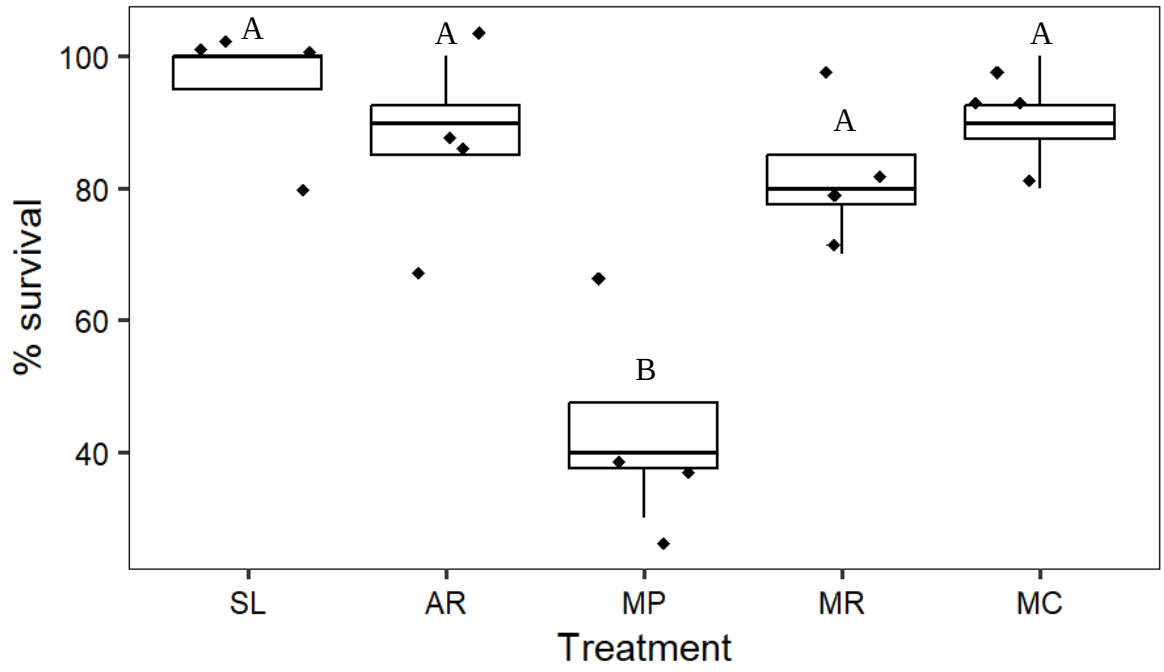

Figure 4. Percent survival of *C. dilutus* exposed to treated and untreated contaminated sediment, control and reference sediments (n=40). Horizontal bars indicate the median, boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range. Treatments identified with different letters (A,B) were determined to be statistically different.

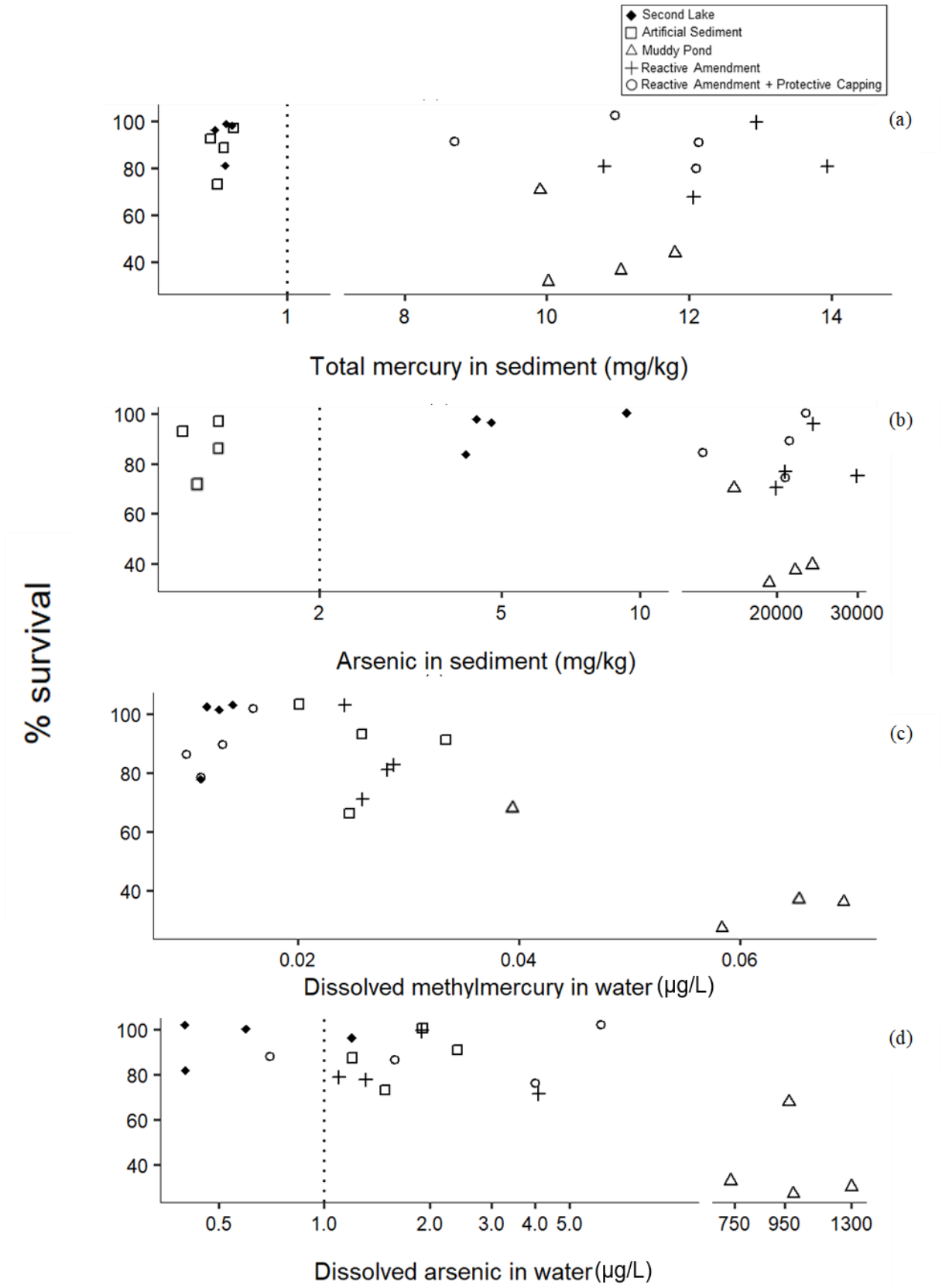

Figure 5. Relationship between the survival percentage of *C.dilutus* larvae at the end of the test and the concentrations of total mercury (a) and arsenic (b) in the sediment and methylmercury (c) and arsenic (d) in the overlying water. The dashed horizontal line represents the procedural detection limit.

chironomid survival at the end of the test. Despite having a lower initial concentration of total mercury compared to MR and MC, and arsenic compared to MR, there was still a significantly lower percentage of chironomids in the MP group that survived (Table 2). By the end of the test, both dissolved methylmercury and arsenic concentrations in the overlying water were significantly lower in MR and MC than the untreated contaminated MP and both treated groups showed significantly higher survival percentages relative to MP (Fig. 5c and 5d). One replicate of the untreated group had a lower concentration (0.039 ng/L) of methylmercury in the overlying water and higher chironomid survival compared to the other replicates (n=3).

The mean head capsule width for chironomid larvae measured at the start of the test was 0.34 ± 0.009 mm (n=20). At the end of the test, the head capsule width of each surviving chironomid in each replicate was measured as an indicator of growth. Larvae exposed to the control (0.571 ± 0.077 mm; n=35) and reference (0.601 ± 0.10 mm; n=38) sediments showed significantly higher mean head capsule widths compared to those exposed to untreated contaminated sediment (0.360 ± 0.075 mm; n=18) (Table 2). Figure 6 indicates that after the

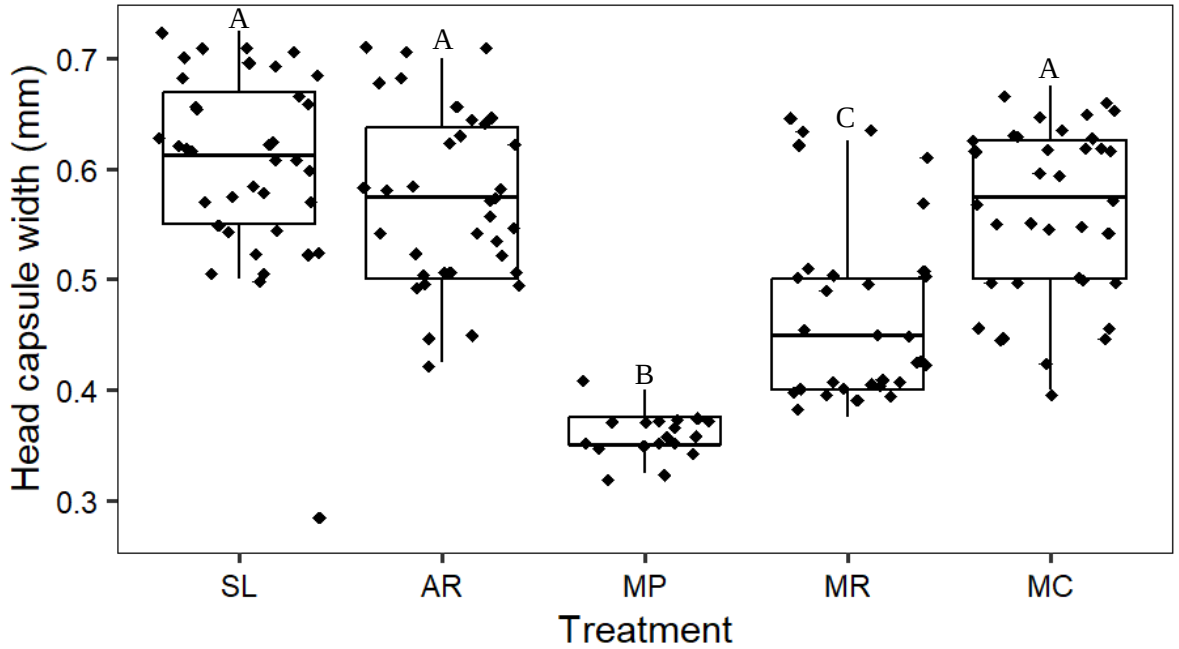

Figure 6. Head capsule widths of *C. dilutus* larvae exposed to treated and untreated contaminated sediment, control, and reference sediments (n=40), indicative of growth during the acute toxicity test. Horizontal bars indicate the median, boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range. Treatments identified with different letters (A,B,C) were determined to be statistically different.

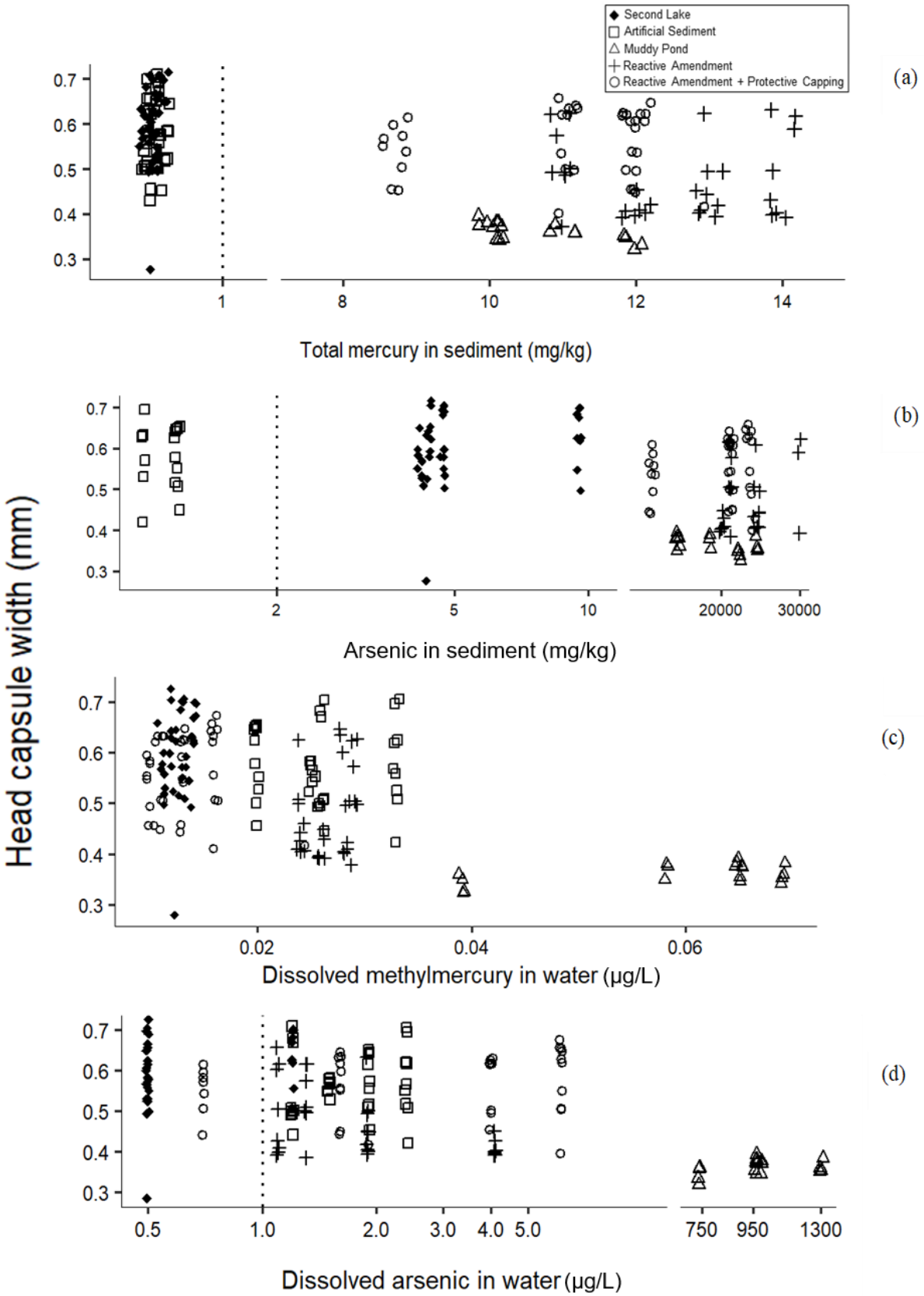

Figure 7. Graphs illustrating the relationship between the head capsule width, indicative of *C. dilutus* growth, at the end of the test and the concentrations of total mercury (a) and arsenic (b) in the sediment and methylmercury (c) and arsenic (d) in the overlying water. The dashed horizontal line represents the procedural detection limit.

contaminated sediment was treated, in groups MR and MC, there was a significant increase in mean larval head capsule widths, measured at 0.474 ± 0.086 mm (n=32) and 0.567 ± 0.019 mm (n=36) respectively compared to the untreated group (Table 2). Furthermore, chironomid growth in the treatment with both the reactive amendment and protective capping (MC) was significantly higher than in the contaminated sediment treated with just the reactive amendment (MR) (Table 2).

Figure 7 illustrates the variation in chironomid head capsule widths between and within different treatments, and how it relates to pre-treatment sediment contaminant concentrations, and dissolved concentrations of contaminants in the overlaying water column at the end of the test . Although the concentration of total mercury and arsenic in the contaminated sediment was very high, exceeding CCME ISQG and PELs, chironomids in the treated contaminated sediments, MR and MP, grew significantly more than chironomids in the untreated MP group (Fig. 7a and 7b). Similarly, when the concentration of dissolved methylmercury (Fig. 7c) or dissolved arsenic (Fig 7d) is higher compared to the other treatments, as in the case of the untreated contaminated sediment, then growth is reduced.

Chironomid larvae surviving at the end of the acute toxicity test were examined for the bioaccumulation of total mercury in their tissue. Although larvae exposed to AR and SL showed relatively lower mercury bioaccumulation (0.74 ± 0.12 mg/kg; n = 35 and 0.14 ± 0.024 mg/kg; n = 38 respectively) compared to the untreated and treated groups, there were no significant differences (Fig. 8). MP chironomids bioaccumulated the highest concentration of mercury (8.54 ± 5.36 mg/kg; n=18) over the course of the toxicity test but it was not significantly higher than bioaccumulation in MR (8.12 ± 2.16 mg/kg; n = 32) or MC (6.37 ± 3.05 mg/kg; n = 36). Although there was no significant difference in bioaccumulation between MR and MC, there was a significantly higher concentration of total mercury in MR sediment at the start of test compared to both MC and MP (Table 2). Despite having a higher concentration of total mercury

in the sediment, chironomid larvae in MR bioaccumulated a lower concentration of mercury in the treated sediments relative to MP (Fig. 9a). Similar concentrations of mercury bioaccumulated in MR compared to MC larvae, even with the latter having the least initial mercury concentrations among all contaminated sediment groups (Fig. 9a). Although the concentrations of dissolved methylmercury in the overlying water at the end of the test was significantly higher in MP compared to the treated contaminated sediment (Table 2), there was no significant difference in mercury bioaccumulation among the treatments (Fig. 9b). One replicate of MP, contained a high concentration of mercury (12 mg/kg) in the sediment compared to the other replicates (n=3) but had the lowest mercury bioaccumulation (0.9192 mg/kg) in larvae exposed to untreated sediment (Fig. 9b). By the end of the test, the overlying water in this replicate also contained the lowest concentrations of dissolved methylmercury (0.039 ng/L) compared to the other MP replicates (Fig. 10b).

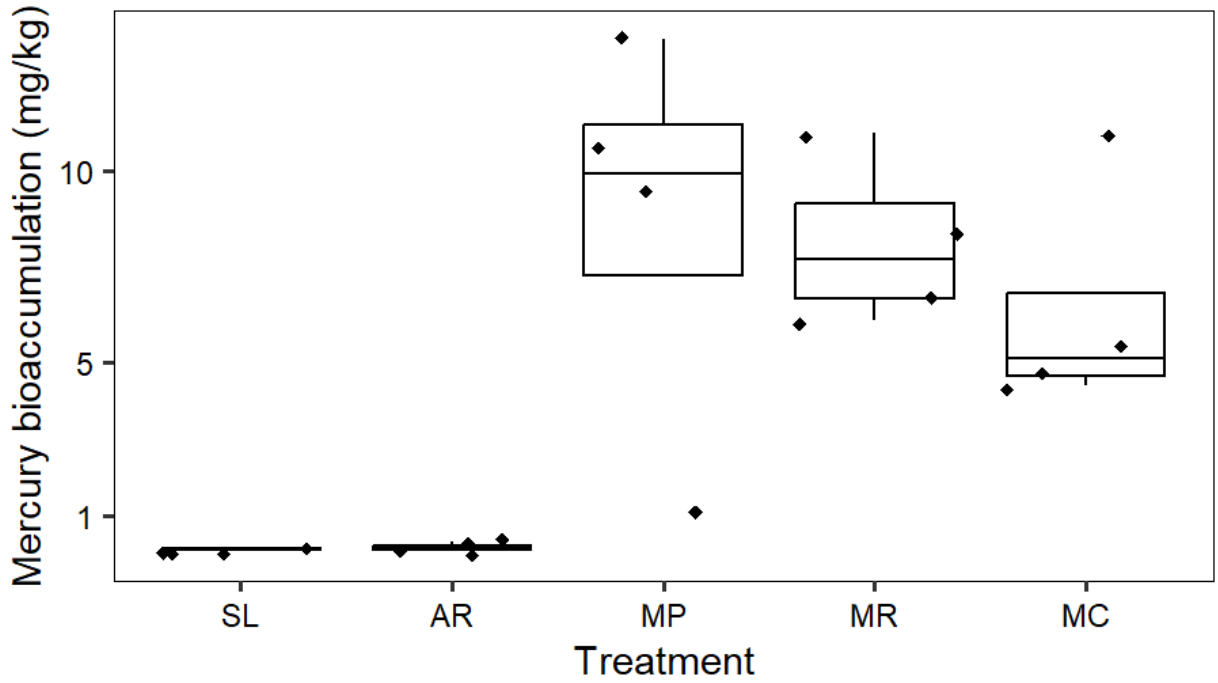

Figure 8. Mercury bioaccumulation in *C. dilutus* larvae exposed to treated and untreated contaminated sediment, control, and reference sediments (n=40), during the acute toxicity test. Horizontal bars indicate the median, boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range. Kruskal-Wallis test revealed no statistical difference between any treatments

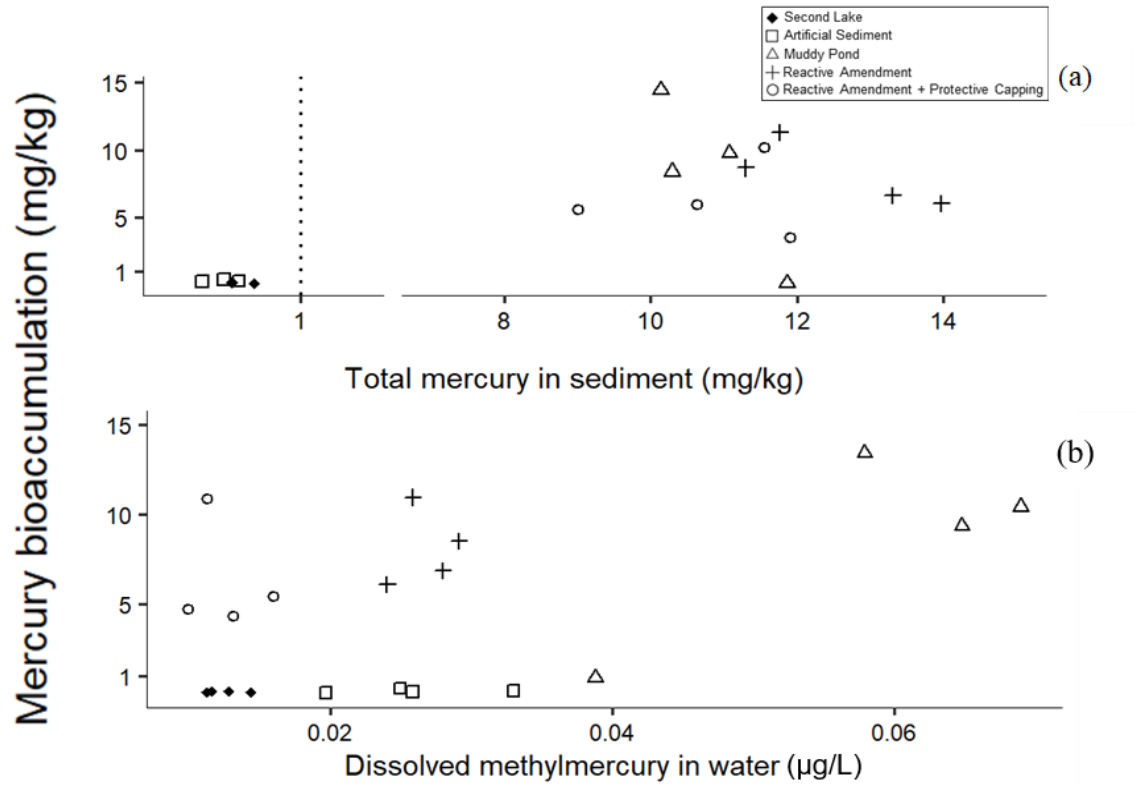

Figure 9. Relationship between mercury (Hg) bioaccumulation in *C. dilutus* tissue at the end of the test and the concentrations of total mercury (a) in the sediment and methylmercury (b) in the overlying water. The dashed horizontal line represents the procedural detection limit.

3.3 Cage Studies

3.3.1 Cage Survival Test

During the 5-day cage study in the laboratory to determine whether the larvae could survive being enclosed in the mini-cages, two chironomids from one of the replicate mini cages with 200 µm mesh managed to escape through a gap in the aquarium sealant. Since these chironomids were not considered for the survival percentage at the end of the test, the sample size decreased from 20 to 18 for one replicate mini cage with 200 µm mesh. There was 100% survival in every mini-cage regardless of mesh size at the end of the test.

3.3.2 Mini-Cage Lab Test

The 5-day laboratory test using sediment and water from Muddy Pond to simulate field conditions, showed that survival significantly differed between 21.6 ± 10.4% (n=20) in mini-cages covered with 300 µm mesh to 78.3 ± 16.1% (n=20) in mini-cages with 243 µm mesh

(Table 2). The mean mini-cage survival with 200 µm mesh was 43.4 ± 25.6%, although it was not significantly different compared to survival in the other mini cages with different mesh sizes. There was no significant difference between the two aquariums holding the mini-cages, in the concentrations of total mercury and arsenic in the sediment samples collected on day 1, and dissolved methylmercury and arsenic in the overlying water from day 5.

3.3.3 Mini-Cage Field Test

A 5-day in situ cage test was conducted at Muddy Pond to determine which mini-cage design allowed for the most exposure to the sediment contaminated with legacy gold mine tailings and resulted in the highest chironomid survival. The initial mean head capsule width of the larvae representative of the culture was 0.35 ± 0.004 mm (n=20). While the test was being conducted, a hurricane passing through the wetland area of Muddy Pond, destroyed some of the cages and killed every chironomid. However, since a few of the chironomids were still contained in the cages, three dead individuals from each cage whose head capsule could be distinguished were collected and measured. The mean head capsule width of chironomids in mini-cages with 200 µm, 243 µm and 300 µm mesh was 0.044 ± 0.004 mm, 0.048 ± 0.003 mm and 0.065 ± 0.003 mm respectively.

Table 2. Differences in test parameters between the different treatments for the acute toxicity test and cage studies. Significant differences ($p < 0.05$) have been specified in the table. One-way ANOVA with pairwise multiple comparison procedure (Holm method) ('A') was used in all comparisons except when normality or equal variance tests failed in which case Kruskal-Wallis one-way analysis of variance on ranks was used with pairwise multiple comparison procedure (Tukey test) ('K').

Test	Matrix	Parameter	Significant difference in treatment	Test	*p* value
Acute Toxicity Test	Sediment	Total mercury	MC>AR	K	<0.001
			MP>AR	K	<0.001
			MR>AR	K	<0.001
			MR>MC	K	0.038
			MR>MP	K	0.002
			MC>SL	K	<0.001
			MP>SL	K	<0.001
			MR>SL	K	<0.001
		Arsenic	MC>AR	K	<0.001
			MP>AR	K	<0.001
			MR>AR	K	<0.001
			SL>AR	K	0.007
			MC>SL	K	<0.001
			MP>SL	K	<0.001
			MR>SL	K	<0.001
	Water	Dissolved methylmercury	AR>MC	K	<0.002
			MP>AR	K	<0.003
			MP>MC	K	<0.004
			MR>MC	K	<0.005
			MP>MR	K	<0.006
			AR>SL	K	<0.007
			MP>SL	K	<0.008
			MR>SL	K	<0.009

			AR>MC	K	<0.009
		Dissolved arsenic	MP>AR	K	<0.001
			MP>MC	K	<0.001
			MP>MR	K	<0.001
			AR>SL	K	<0.001
			MP>SL	K	<0.001
			MR>SL	K	<0.001
			MC>SL	K	<0.001
	Chironomid	Survival	AR>MP	A	0.001
			MC>MP	A	0.001
			MR>MP	A	0.005
			SL>MP	A	<0.001
		Growth	AR>MP	K	<0.001
			MC>MP	K	<0.001
			AR>MR	K	0.002
			MC>MR	K	0.002
			MR>MP	K	0.005
			SL>MP	K	<0.001
			SL>MR	K	<0.001
Mini-cage Test	Chironomid	Survival	243µm>300µm	A	0.02

4.0 Discussion

In this study, benthic chironomid larvae were exposed to treated and untreated wetland sediment highly contaminated with mercury and arsenic to determine whether the reactive amendment and protective capping layers were safe for the sediment-dwelling invertebrates and if the toxicity of the tailings and bioaccumulation of mercury at contaminated wetlands could be reduced. From the analyses of the overlying water at the end of the test, it was determined that the contaminated sediment in the MR group treated with only the reactive amendment (micro-sized ZVI) had significantly lower dissolved methylmercury and arsenic in

the water column compared to the untreated Muddy Pond sediment. This is consistent with Chapman et al. (2020) who evaluated the use of nZVI at different concentrations (0%, 2%, 4% and 8%) as an amendment for Muddy Pond sediment with a 28-day test and determined that it was successful in limiting the mobility of both mercury and arsenic from the highly contaminated sediments into the water. In comparison, their untreated sediment samples from Muddy Pond generally contained higher concentrations of arsenic compared to the sediment used in this study (29566 ± 3449 mg/kg vs 23750 ± 4500 mg/kg) but lower concentrations of total mercury (10 ± 0.3 mg/kg vs 12.5 ± 1.29 mg/kg). Despite these variations in concentrations, ZVI is capable of limiting the mobility of mercury and arsenic from the wetland sediment into the water column.

With the addition of the protective capping layer to the contaminated sediment treated with ZVI in MC, there was a significant decrease in methylmercury concentrations compared to MR. This indicates that the protective capping layer further improves the efficacy of the reactive amendment to limit contaminant mobility. This can be explained by the components in the protective capping layer; zeolite which promotes surface complexation, cation exchange, and precipitation of mercury, and sand which supports the stabilization of the active capping material (Bailon et al., 2020; Shi et al., 2009). In a recent study, Chaudhary et al. (2022) tested the use of only a capping layer composed of sand and zeolite as a technique for remediating total mercury and methylmercury in the Hyeongsan River estuary in South Korea. Their results showed a 56% and 34-41% reduction in total mercury in the sediment and methylmercury in porewater respectively, after being treated for two months. Many such field experiments (He et al., 2015; Hong et al., 2019) have demonstrated similar results, indicating that the results of the current study are in agreement with other research. This indicates that the protective capping layer is effective in reducing mercury mobility, and therefore its bioavailability to aquatic organisms. There was no significant difference in the arsenic concentrations in the

water column between MR and MC since ZVI in the reactive amendment, which was added to both treatment groups, is the only substance that adsorbs arsenic. However, arsenic concentrations in the water overlying both MR and MC were significantly reduced compared to MP. Therefore, the use of the protective capping layer significantly aided the reactive amendment in limiting the mobility of mercury from the sediment to the water, as well as arsenic but not to the same extent. Higher concentrations of the treatments may further decrease contaminant mobility, but could potentially become toxic to benthic organism colonizing these contaminated areas.

The results of the reference toxicity test conducted prior to the acute sediment toxicity test were as expected, with lower survival percentages for larvae exposed to higher concentrations of NaCl and no deaths in the control. This response to spiked water, indicates that the results for subsequent toxicity testing using the laboratory reared larvae are accurate and reliable. Furthermore, the larvae used were not overly sensitive and therefore fit for toxicity tests with mercury and arsenic (Environment Canada, 1997). Chironomids exposed to untreated sediment from Muddy Pond during the acute sediment toxicity test had a significantly lower survival percentage and growth compared to those in treated contaminated sediment. This was expected since the concentration of dissolved arsenic in the overlying MP water far exceeded the no-observed-effect concentration (NOEC) for growth, determined by Liber et al. (2011) to be <0.42 mg/L. Survival percentage and growth in MR and MC exceeded MP by ~1.5 times, with chironomids in these treated groups appearing to be more active and robust, indicating that the reactive amendment and protective capping layers are effective in reducing the toxicity of the tailings in Muddy Pond sediment. Furthermore, growth in MC was significantly higher than in MR and this can be explained by the presence of the protective capping layer in MC which is more habitable than the ZVI layer and provides sand particles for chironomid larvae to create shelters in the form of tube-like casings. In situations where

larvae within the sediment are unable to obtain sufficient oxygen, they create tubes extending beyond the length of their body to accumulate higher concentrations of dissolved oxygen from the upper layers of the water column (Modak et al., 2022). In this study, larval casings in MR and MC were relatively small and approximately the length of their body. This suggests that the application of the treatments generally do not create hypoxic environments within the sediment and are acceptable for chironomid larvae. Moreover, larvae were observed to incorporate the protective capping material into their tubing, further indicating that the treatment is safe for *C. dilutus*.

The absence of significant differences in mercury bioaccumulation between the treated and untreated Muddy Pond groups can be attributed to the variation in the initial concentrations of total mercury in the sediment due to bioturbation and natural hydrodynamic processes (Bailon et al., 2020) and low larval sample mass due to limited growth in the untreated group. The chironomids in the highlighted MP replicate had the lowest mercury bioaccumulation even though the sediment contained the highest concentration of mercury compared to the other replicates. Since the concentration of methylmercury in the water column of that MP replicate was the lowest in the MP group, it is likely that the pathway for bioaccumulation of mercury in chironomids is through the overlying water. This is consistent with Jonsson et al. (2022) who injected mercury tracers in the water column and sediment and concluded that mercury in chironomids bioaccumulated to a larger extent through the overlying water. Since this study demonstrated the ability of the reactive amendment and protective capping to limit the mobility of mercury to the overlying water, it is likely that future studies that utilise contaminated sediment with relatively similar concentrations of mercury in treated and untreated groups, will observe significant differences in bioaccumulation.

Results from the cage survival test indicate that chironomid larvae are capable of living within the enclosures at least for a 5-day exposure period, regardless of the mesh size used and

there is minimal to no stress induced within the organisms that could impact *in situ* cage studies in the future. The goal of the mesocosm test at Muddy Pond is to evaluate how treatments will impact chironomids in situ under natural conditions, as opposed to the lab acute toxicity test which was conducted in a controlled environment. Since differences in mercury bioaccumulation between treated and untreated mesocosms will be evaluated, organisms must be alive at the end of the test to allow for adequate gut purging and rinsing so that only the contaminant within the tissues is measured. Therefore, 243 µm was identified as the ideal mesh size for cage studies at Muddy Pond as it allowed for the highest survival at the end of the mini-cage lab test, without allowing chironomids to escape.

This study demonstrated that the reactive amendment and protective capping layers are safe for *Chironomus dilutus* larvae and reduce the toxicity of the tailings and bioavailability of mercury in contaminated wetland sediment. The field mesocosm experiment at Muddy Pond in the summer of 2024 will examine the durability of the treatments under weather-related disturbances and erosion as well as the changes in nutrient content after the addition of the treatments.

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