MUNICIPAL MUSSELS: AN INVESTIGATION OF THE MUTUAL BENEFITS OF FRESHWATER MUSSEL PRESENCE IN A MUNICIPAL WATER SUPPLY

by

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Dalhousie University is located in Mi'kma'ki, the ancestral and unceded territory of the Mi'kmaq. We are all Treaty people.

Dedication

This thesis is dedicated to Kellie White, whose friendship, generosity, patience, and mentorship made the entire thing possible.

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Abstract

Freshwater mussels are a keystone group in lake ecosystems, including some surface water supply sources utilized by Cape Breton Regional Municipality (CBRM) Water Utility. My research aims to establish the value of ecosystem services provided by freshwater mussels to the water treatment process, including their potential to reduce water treatment costs by reducing the need for chemical and mechanical treatments. Experiments were conducted comparing metal concentrations over time in tanks containing freshwater mussels and those without. The results showed significant differences in metal concentrations associated with mussel filtration. Local lakes with mussels were also found to have significant differences in metal concentrations and other water parameters, higher water treatment costs, and overall higher water quality drinking water than those without mussels. These results highlight the impact of freshwater mussels on water quality and their potential application as biofilters in pre-treatment for tap water and will inform future decisions by CBRM Water Utility regarding policy, conservation, climate change adaptation, and water security. Freshwater mussels have the potential to mitigate water-related risks, making them a critical component in the effort to build a more resilient water system in the Cape Breton Regional Municipality.

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Chapter 1: Introduction

The availability of liquid water on Earth is considered to be one of the two requirements for the genesis and continuance of life as we know it, the other being the availability of organic molecules (Bada, 2004). Of all the surface water on Earth, only 2.5% - 3% is freshwater suitable for drinking, and less than 1% of that freshwater is liquid and easily available (Rijsberman, 2006; Ahuja, 2009; Vasistha and Ganguly, 2020). Access to high quality drinking water is essential to human health and wellbeing, and the integrity and health of natural ecosystems is a major component of sustainable freshwater sources (Li and Wu, 2019; Zhang *et al.*, 2023). Human activity and its interactions with natural processes has led to decreases in water quality for much of the world's surface water resources, through climate change, sedimentation, eutrophication, and contamination with various pollutants (Vasistha and Ganguly, 2020; Zhang *et al.*, 2023).

Bivalves such as freshwater mussels (hereafter referred to as mussels) and other suspension feeding invertebrates play an important role in these freshwater ecosystems and contribute to the ecological stability and water quality of these systems (Vaughn and Hakenkamp, 2001; Vaughn, 2018). However, freshwater mussels are increasingly disappearing from lakes, rivers and streams. Up to 75% of the freshwater mussel species in North America are in decline, suffering losses from such threats as habitat destruction, climate change, pollution, and introduction of invasive species (Lydeard *et al.*, 2004; Haag and Williams, 2014; Ferreira-Rodriguez *et al.*, 2017). Global conservation efforts have increased in the past 30 years, with increased research interest, development of standardized research and conservation protocols, habitat restoration projects, breeding and translocation projects and legal protections for particularly threatened species (Williams *et al.*, 1993; Haag, 2012; Lopes-Lima *et al.*, 2017; Zieritz *et al.*, 2017; Sousa *et al.*, 2022).

Sources of pollutants in freshwater lakes and waterways are varied. Major anthropogenic sources of dissolved and particulate metals include industrial activity such as mining, manufacturing, agriculture, and waste disposal practices (Zhou *et al.*, 2020). Natural weathering of metal-containing rocks and soils can also be exacerbated by climate change (Frings, 2019; Houle *et al.*, 2020; Belyazid *et al.*, 2022). Metal concentrations in water bodies can accumulate over time after atmospheric deposition or land-based sources (ie: runoff) due to evaporation (Winchester and Nifong, 1971; Norton, 2007; Roberts *et al.*, 2019; Bakshi *et al.*, 2023). Nutrient enrichment (eutrophication) is another major factor in water quality deterioration and is primarily caused by increased levels of nitrogen and phosphorus from untreated sewage, agricultural practices (ie: soluble fertilizers), and climate change (Le Moal *et al.*, 2019; Rozemeijer *et al.*, 2021). These pollutants and processes can have complex interactions which are detrimental to freshwater mussel health and survival, as well as negatively affecting the safety and treatment costs of drinking water for humans (Vasistha and Ganguly, 2020; Bakshi *et al.*, 2023). Therefore, mussels and humans have a shared stake in lake water quality. In order to demonstrate the contribution of mussels to this mututally beneficial relationship, I decided to investigate the role of freshwater mussels in human wellbeing by identifying ecological services provided by freshwater mussels.

What are ecological services?

For many decades, researchers and policy makers have increasingly come to the understanding that the benefits of natural systems have real, measurable impacts on human economies and the well-being of individuals and communities (Alcamo *et* *al.,* 2003). These ecological services are derived from natural assets, and there are now several accepted theoretical frameworks which allow for estimation and quantification of the monetary benefit of the presence of the resources, organisms, populations, and ecological interactions within a given environment (Alcamo *et al.,* 2003; TEEB, 2010; Saarikoski *et al.*, 2016; Meraj *et al.*, 2021). The results of these inquiries and estimates are often intended to contribute to policy decisions, management and conservation of natural assets, as well as decrease service delivery costs, resulting in a system which prioritizes sustainability, investment and longevity of human industry (Alcamo *et al.,* 2003).

The acknowledgement that humans are a part of the health and well-being of the environment, and the idea that pristine natural conditions are important to human life are nearly as old as recorded history (Gómez-Baggethun *et al.*, 2009). The modern concept of "ecosystem services" was introduced in the second half of the 1970s, as environmentalists worked to increase public understanding, acceptance and support of conservation efforts (Westman, 1977; Gómez-Baggethun *et al.*, 2009). As environmentalism increased in popularity and scope in the following decades, the idea of ecosystem services also became more popular. The Millennium Ecosystem Assessment (Alcamo *et al.,* 2003) was a major milestone which brought ecosystem services and their valuation into the purview of policy makers in North America, and since then, several conceptual frameworks have been adopted by governments and regulatory bodies in a number of countries around the world (TEEB, 2010; Haines-Young and Potschin-Young, 2018). The United Nations has adopted the CICES (Common International Classification of Ecosystem Services; Haines-Young & Potschin-Young, 2018) as the preferred theoretical framework by which to classify the different types of ecological services, described below.

From a socioeconomic perspective, humans are putting increasing demands on the finite resources of our planet, and population growth and anthropogenic climate change are increasing the rate of resource depletion, habitat destruction, and biodiversity loss (Altieri & Gedan, 2014; Cavicchioli *et al.*, 2019; Gonçalves-Souza *et al.*, 2020; Cowie *et al.*, 2022). Humanity, and all life on Earth, is dependent upon the resources provided by the biosphere for survival, so it is imperative that this current trajectory is reversed. Ecological footprint analysis has conservatively estimated that at least three-to-five Earth-sized planets would be required to provide the resources to support the human population if everyone lived like the average Canadian (Rees and Wackernagel, 1997; Wackernagel *et al.*, 2006; Footprint Data Foundation, 2022). Furthermore, Hardin's (1968) ecological theory of the "tragedy of the commons" shows that intervention from government is necessary to prevent the exploitation of the natural world. The framework of ecological services allows policy makers and economists to use evidence-based concepts to inform decisions about land-use, resource extraction, and other activities which may impact the integrity of a natural area (Daly & Farley, 2011). Incorporating ecological services into our economic system is intended to enable policy makers to conceptualize the value, complex interactions and processes within an ecosystem, and is a persuasive tool for policymakers who are often unaware of current ecological theory paradigms, providing evidence and support for policies that protect or enhance ecological systems by showing the costs incurred by their loss or deterioration (Daly & Farley, 2011). There are some difficulties associated with this approach, however, since some ecological services, such as pollination, cannot be replaced through human technology if lost (Fisher and Turner, 2008). Furthermore, many systems include large information gaps – using the same example, it is possible to overlook services provided by pollinators that are not directly related to agriculture (such as pollination of wildflowers), but which may also directly or indirectly provide value to humans (Fisher and Turner, 2008; Hanley *et al.,* 2015; Majewski, 2018).

As ecological services gradually gained more traction with policy makers, new, formal approaches to addressing problems like anthropogenic pollution began to emerge, such as "cap-and-trade" systems, incentives for industrial good behaviour, and penalties for environmental misdeeds. However, in the context of modern economies with strong incentives to favour short term gains over sustainability, there is often difficulty in achieving the intended results (Bayon, 2004; Guerry *et al.*, 2015; Cushing *et al.*, 2018). For example, in the United States, amendments to the Clean Air Act implemented a system by which industrial polluters were allotted a certain number of sulfur dioxide "credits" (ie: specified amounts of sulfur dioxide they were allowed to release into the atmosphere), which could be bought, sold or traded (Stavins, 1998). This approach was considered to be successful, and emissions did reach the targeted reductions following its implementation (Stavins, 1998; Bayon, 2004), however, other programs intended to reduce greenhouse gases have been less demonstrably effective, or ineffective entirely (Bayon, 2004; Cushing *et al.*, 2018). A similar system of trade-off style economics was used in order to allow a certain amount of industrial destruction of wetlands in the U.S., in exchange for preservation, establishment or remediation of other wetlands (Bayon, 2004). As other researchers have noted, the way this policy was implemented meant that a market developed for the sale of wetland destruction, basically, and environmental economists were able to determine the monetary "value" of wetland per acre (approximately $44,000/acre in the year 2000) by calculating the average transactions that were carried out by land developers (Bayon, 2004). In Nova Scotia, altered wetlands must be replaced in a 2:1

ratio, with 2 acres of wetland restored for every acre that has been altered (Government of Nova Scotia, N.D.). There is considerable debate about whether this method of valuation of ecosystem services is ultimately beneficial, however, since in practice it becomes something like purchasing the "permission" to destroy wetlands (Bayon, 2004). Habitats may be irreplaceable, restoration and conservation of other wetlands may not provide the same services as the destroyed land, size of wetland does not necessarily determine the amount of services provided, and there are economic incentives to spend the least amount of money and effort in these efforts (Roberts, 1993; Bayon, 2004; Heberling *et al.*, 2010; Womble and Doyle, 2012; Tillman and Matthews, 2023).

The United Nations has adopted the CICES (Common International Classification of Ecosystem Services; Haines-Young & Potschin-Young, 2018) as a common framework by which to classify the different types of ecological services, and this is the primary framework which I will be using for this thesis. This framework presents a general overview of the types of services ecosystems can provide, breaking them down into four major categories, and relating them to human wellbeing (Figure 1). The four main categories in this framework are Provisioning, Cultural, Regulating and Supporting. My focus will primarily be the latter two categories. Another aspect of the CICES framework is that is presents different concepts of valuation, including economic use values, as well as non-use values (Figure 2).

The CICES framework has been used in varied contexts and has significantly enriched the perspective of researchers and policy makers. For example, there have been comprehensive reviews of major ecological systems such as marine and freshwater plankton, demonstrating their cultural value, and their role in the provision of oxygen, primary production, nutrient cycling, climate regulation, and provision of ingredients for products made by humans (Naselli-Flores and Padisak, 2022; B-Beres *et al*., 2023). Freshwater macrophytes have also been reviewed in this context, demonstrating the provisioning of food, food additives, fibre, habitat for aquatic organisms, oxygen production, nutrient cycling, and cultural importance (Thomasz, 2021). Another example of comprehensive ecosystem services research has reviewed the services provided by neotropical fishes, which are recognized for their role in provisioning humans with food, but also provide value as bioindicators, ornamental and recreational species, nutrient cycling, ecosystem engineering, top-down control of aquatic food webs, and even seed dispersal (Pelicice *et al.,* 2022).

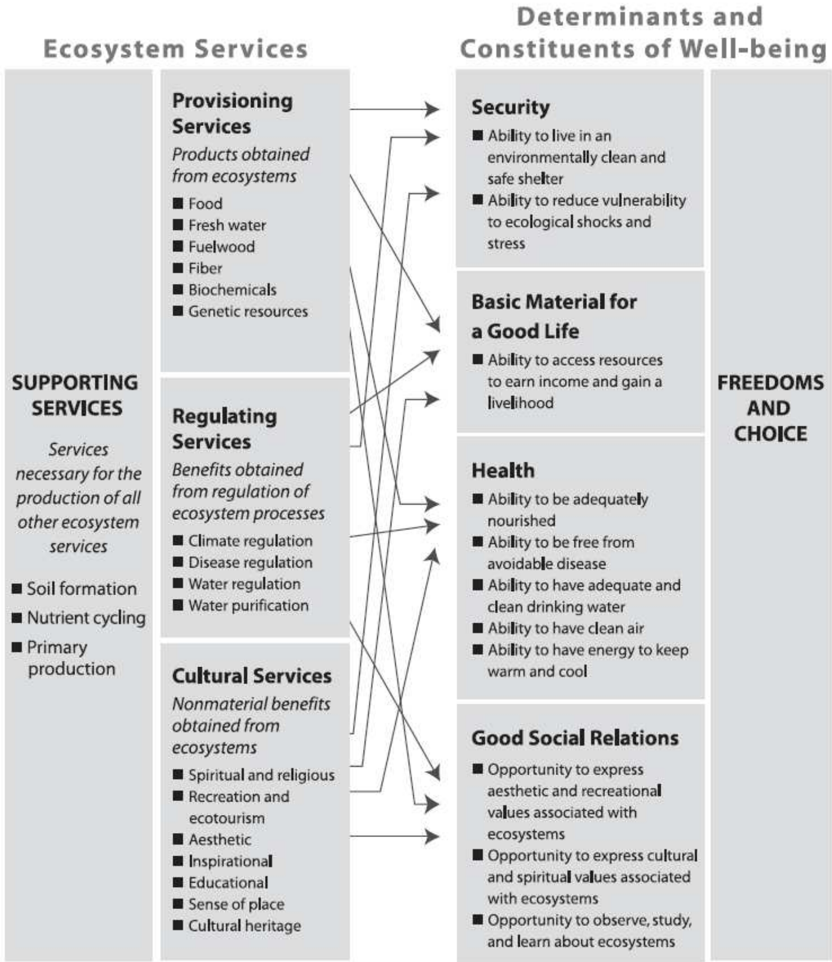

Figure 1. Ecosystem services and their connections to human wellbeing. From Alcamo *et al.* (2003).

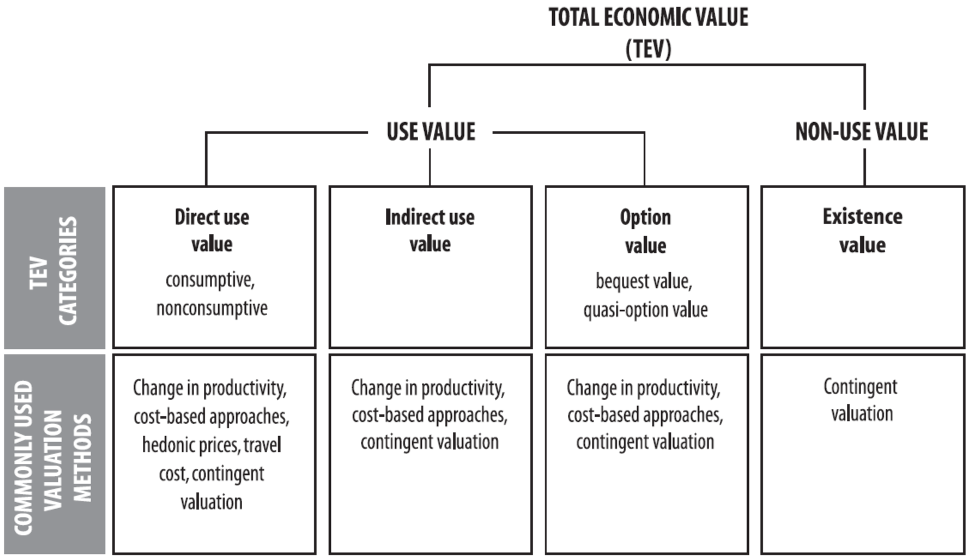

Figure 2. Categories and valuation methods for ecological services. From Alcamo *et al.* (2003).

Provisioning services

Provisioning services refer to the use of organisms or their byproducts directly for food, building materials, ornamental resources, or for energy. Freshwater mussels have historically been used as a food source in certain North American indigenous traditions, and are currently an important food source in some communities in Asia and Africa (Haines-Young & Potschin-Young, 2018).

The value of provisioning services of freshwater mussels is possible to measure, due to the existence of primarily historical records, although illegal harvesting takes place in some areas where mussels are protected, and therefore records may be somewhat inaccurate (Cosgrove *et al.*, 2016). In North America, the majority of records relate to the catch and sales of mussel shells for use in the pearl and pearl button industry. This industry began in the mid-19th century, and good records exist which allow us to estimate the monetary value of not only the catch and products produced, but also the resulting loss of income from the industry as mussel populations declined across much of the continent (Anthony and Downing, 2001).

Cultural services

Cultural services include values that humans ascribe to the experiential, spiritual, cultural, or aesthetic properties of ecosystems and organisms. This division of services can be difficult to estimate in terms of monetary value, due to its subjective nature and potential for temporal change as cultural values and trends change over time. However, some techniques of measurement have been employed to estimate these values, such as the measurement of nearby property values before and after damage or restoration of an area, or stakeholder interviews about personal opinions on the value of an ecosystem and its perceived integrity (Hernández-Morcillo *et al.*, 2013; Milcu *et al.*, 2013; Hirons *et al.*, 2016).

The Truth and Reconciliation Commission of Canada (TRCC; 2015) has provided 95 calls to action which are meant to rebuild trust between settlers and Indigenous peoples in Canada, including several which are particularly relevant to researchers in biology, ecology and other natural sciences (Wong *et al.*, 2020). One such call to action is for natural science researchers to understand the cultural and socio-political significance of their research sites, and another calls for recognition that generating ecological knowledge is a shared goal of both Indigenous peoples and settlers (Truth and Reconciliation Commission of Canada, 2015; Wong *et al.*, 2020). One method of addressing some of these calls to action is employing the concept of Two-Eyed Seeing, a guiding philosophical principle of Integrative Science brought forth by Mi'kmaw Elder Albert Marshall (Marshall *et al.*, 2010; Bartlett, 2011). The indigenous worldview focuses on the relationship between humans and nature and our participation in natural processes, rather than conceptualizing humans operating outside of the ecosystems (Hatcher et al., 2009). In Two-Eyed Seeing, Indigenous knowledge and the perspectives of Western science are woven together, which both enriches understanding and fosters community and collaboration between Aboriginal and non-Aboriginal peoples, allowing us to tackle the complex problems of environmental stewardship with the perspective of our own role in the ecosystem, rather than viewing ourselves as insulated from nature (Marshall *et al.*, 2010; Bartlett, 2011; Kutz and Thomaselli, 2019). Thus, the study of freshwater mussels and their connection to the health of waterways, incorporating Indigenous knowledge and management perspectives has significant cultural value due to its potential contributions to the ongoing work of reconciliation.

Regulating Services

Regulating and supporting services comprise the main focus of this research, as I attempt to frame the most easily quantifiable services provided by freshwater mussels within the context of the CBRM Water Utility. Regulating services relate to the mediation and control of waste, toxins, air and water flows, as well as the maintenance of environmental parameters (Haines-Young and Potschin, 2018).

The obvious contribution made by freshwater mussels in the context of regulating services relates to improving water clarity and (Figure 3). In order to feed on phytoplankton and other microscopic organisms, freshwater mussels actively filter 0.5 - 1L/h of water across their gills' mucous membranes, entrapping suspended particles (Vaughn, 2008). These particles are transported via ciliary action to a sophisticated sorting organ, where fleshy palps selectively direct particles to the stomach or excurrent siphon based on size and composition - ultimately being digested and excreted or encased in mucous and expelled as pseudofeces, respectively (Haag, 2012). Therefore, the filtration activity of freshwater mussels is responsible for reducing turbidity in the water column by removing silt and inorganic particles as well as plankton and bacteria. Research has also shown that some species of mussels may even remove cyanobacteria from the water column, potentially mitigating harmful algal blooms (HABs) (Waajen *et al.*, 2016). This action is particularly important as cyanobacterial HABs are predicted to increase as climate change progresses over the next several decades (Scholes, 2016; Chapra *et al.*, 2017; Gobler, 2020). The Federation of Canadian Municipalities has created the Municipalities for Climate Innovation Program (MCIP), which has identified the management of natural assets as a strategy for increasing climate resilience (Federation of Canadian Municipalities, 2023). Freshwater mussel populations could be an important natural asset in management of water resources, both for human consumption and ecological integrity.

Other research has shown that freshwater mussels can mitigate the effects of eutrophication and runoff (Waajen *et al.*, 2016; Chowdhury *et al.*, 2016), reduce pharmaceuticals in water (Faust *et al.*, 2009; Ismail *et al.*, 2014; Othman *et al.*, 2015), and even reduce concentrations of pathogenic bacteria such as *E. coli* (Raikow and Hamilton, 2001; Haag, 2012; Ismail *et al.*, 2015).

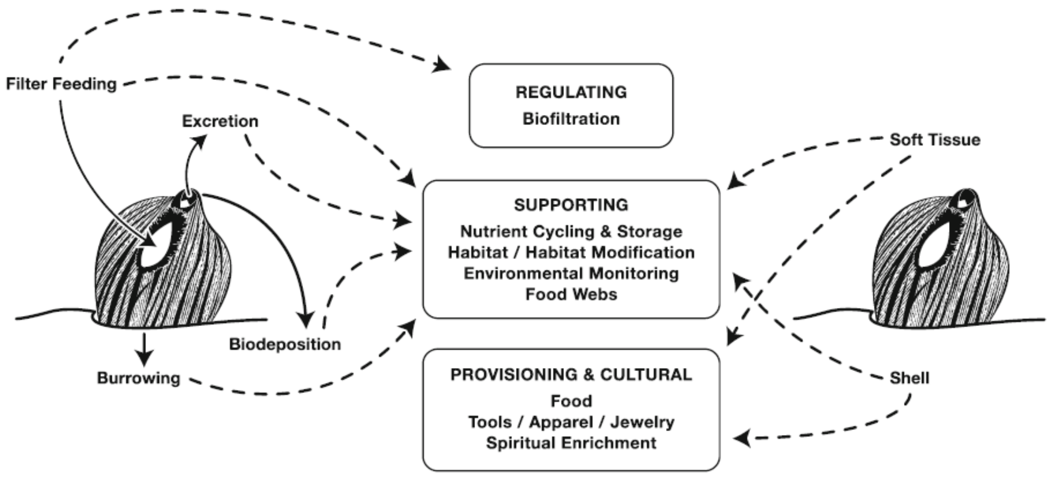

Figure 3. Ecosystem services provided by freshwater mussels and their tissues. From Vaughn (2018).

Supporting services

Supporting services provided by freshwater mussels can be more difficult to quantify. These are services that influence local ecosystems, often indirectly, in a way which affects human life. Research has shown that freshwater mussels can influence micro-currents near the interface between sediment and the water column which can provide habitat for small organisms such as invertebrates and fish (Strayer *et al.*, 1994; Beckett *et al.*, 1996; Haag, 2012; Figure 3). By altering erosion rates and currents in bodies of water, mussels can change the patterns of sediment settlement and energy regimes of a stream or river (Allen and Vaughn, 2011). There may even be ramifications for erosion and river migration patterns, downstream effects, or the recruitment of conspecifics or other organisms (Beckett *et al.*, 1996). Over time, these effects can gradually build to significant changes in the environment, which, along with their filtration and clarifying action in the water column, is why freshwater mussels are often framed as ecosystem engineers or keystone species (Geist, 2010; Darrigran and Damborenea, 2011; Chowdhury *et al.*, 2016; Bolotov *et al.*, 2018; Emery-Butcher *et al.*, 2020).

Freshwater mussels have a hard, calcified shell, which itself can act as a substrate for various epibionts (organisms which live on other organisms), which themselves may influence the environment around them (Gutierrez *et al.*, 2003; Haag, 2012; Vaughn, 2018). In addition to clarifying the water column, the removal of particles and subsequent light penetration alters the assemblage of plankton and macroscopic primary producers in an aquatic system (Chowdhury *et al.*, 2016; Vaughn, 2018). This in turn will affect the food web and the associated trophic interactions in and near the waters in which they live.

Freshwater mussels have long been used by humans as bioindicators of heavy metal contamination due to their ability to bioaccumulate these impurities (Tynan *et al.*, 2005; Sohail *et al.*, 2016; Xu *et al.*, 2022). Mussel shells represent a long-term storage for impurities, isotopes and, much like tree growth rings, their growth patterns can provide a record of environmental conditions during mussel development, from prehistoric to modern times (Blazejowski *et al.*, 2013; Fritts *et al.*, 2017). Mussels have been used as bioindicators of fluctuations in concentrations of metals such as Silver, Arsenic, Cadmium, Cobalt, Copper, Chromium, Nickel, Lead, Selenium and Zinc (*eg.*: Jamil *et al.*, 1999), along with larger scale pollution events of contaminants like mercury (Brown *et al.*, 2005). Mussel soft tissues can be used as biomonitors for shorter term fluctuations, and their propensity to bioaccumulate certain contaminants (such as pharmaceuticals) in soft tissues can provide information about local and upstream conditions (Du *et al.*, 2014; Vaughn, 2018). Tissue sampling typically requires destruction of mussels (*eg.*: Jamil *et al.*, 1999), and therefore tissues of imperiled species are not suitable for use as bioindicators. Due to the sensitivity of juvenile mussels to water quality parameters such as nitrogen loading, the presence and density of juveniles can also be used to qualitatively assess the condition of a water body (Newton & Bartsch, 2007).

Research Aims and Objectives

The central aim of this thesis research was to characterise the ecological services provided by freshwater mussels in relation to the municipal water supply in Cape Breton, Nova Scotia, and to demonstrate the mutually beneficial relationship between freshwater mussels and humans. To this end, I have assessed the impact of freshwater mussels on the quality of surface water supplies through an examination of differences in water chemistry associated with the presence or absence of freshwater mussels and the nature of those relationships.

This is a manuscript-based thesis with two manuscripts. The first manuscript in Chapter 2 begins with a brief description of the discovery of Yellow Lampmussel (*Lampsilis cariosa* (Say, 1817)), a species of “Special Concern” (federally) and designated as “threatened” within Nova Scotia (Fisheries and Oceans Canada, 2010; Nova Scotia Department of Natural Resources and Renewables, 2022), which was discovered in 2012 in a drinking water source lake (Pottle Lake, serving North Sydney and surrounding areas) in the Cape Breton Regional Municipality (CBRM) Water Utility (White, 2015; A. Mazzocca, personal communication, November 2021). The interest in this rare species by the CBRM Water Utility and the local community led to a number of research programs about *L. cariosa* and freshwater mussels in general by local investigators (White, 2015; White 2016; K. White, personal communication, May 2016). One of the outcomes of the initial exploratory research was the discovery that the water quality in Pottle Lake was very high, and treatment costs very low in comparison to some other sites (A. Mazzocca, personal communication, November 2021). Chapter 2 continues with a literature review on the nature of ecological services, a framework chosen in an effort to contextualize the role of freshwater mussels in the CBRM Water Utility. I specifically focus on the regulating and supporting services provided by freshwater mussels.

Research question 1

Are water quality and treatment costs in CBRM Water Utility lakes related to the presence of freshwater mussels?

In consideration of the high water quality of Pottle Lake and the context of ecological services provided by freshwater mussels, it was logical to hypothesize that the presence of freshwater mussels might be positively correlated with water quality in the CBRM Water Utility system. Subsequently, I investigated a preliminary case study of the municipal water utility in the Cape Breton Regional Municipality and the costs associated with water treatment for source water containing mussels compared to sources without mussels. The methods for this analysis are as follows. Four water treatment facilities which utilize surface water sources in the CBRM Water Utility were selected for analysis, representing three methods of drinking water treatment (Figure 1). Two of the lakes (Pottle Lake and Waterford Lake) contain populations of freshwater mussels, while two (MacAskills Reservoir and Kelly Lake) do not have mussels (White, 2015; White 2016). The yearly costs for chemicals (excluding chlorine and fluoride) and equipment maintenance were divided by the average daily flows during that year, to calculate a snapshot cost-per-unit of water treated (in this case, per 10 000 imperial gallons). Data from quarterly monitoring assessments of metal concentrations provided by the CBRM Water Utility were also analyzed to compare metal concentrations in raw and treated water from each of the four treatment facilities, to determine if there were differences between water from each type of lake.

<u>Research question 2</u>

Are there consistent differences between mussel and non-mussel lakes throughout CBRM?

The results of this snapshot investigation into CBRM water treatment facilities led to the question of whether correlations found within these four lakes should be consistent among a large selection of mussel and non-mussel lakes within Cape Breton County. Therefore, an observational study was designed to measure and compare lakes with and without mussels. Thirteen lakes were selected for analysis, 8 containing mussels, and 5 without. Surface water samples (general chemistry = 250ml bottle, nutrients = 250ml bottle with H_2SO_4, and metals = 120ml bottle with HNO_3; Bureau Veritas, 2023) were collected from each lake in November 2020, October 2021, and July 2022, and sent to a commercial laboratory (Maxxam Analytics / Bureau Veritas, CBRM) for testing.

RCAp-MS analysis was done on all water samples collected from study lakes to measure 47 parameters, including major ions, 23 metals, and other physical-chemical characteristics (ACWWA, 2004). This is the method used by the CBRM Water Utility, and therefore it was used in this research to compare with data sets previously collected by CBRM if required, and for use in internal reports in the future (ACWWA, 2004; A. Mazzocca, personal communication, September 2021). Two-way Analyses of Variance were conducted using Minitab, version 21 (Minitab, 2010) to statistically compare water quality parameters between mussel and non-mussel lakes.

Research question 3

Are freshwater mussels the cause of differences in water quality?

The observational studies in Chapter 2 were intended to provide support for the hypothesis that freshwater mussels are correlated with increased water quality in lakes and therefore to decreased treatment costs, however, it is not possible to determine from these observations which or to what degree the differences observed were due to the presence and activity of freshwater mussels. The second manuscript in Chapter 3 describes a randomized treatment + control tank experiment designed to demonstrate the causal relationship between water chemistry (specifically in relation to metal concentrations) and freshwater mussel presence, whether by active mussel filtration or incorporation into body tissues.

The methods for the tank study are as follows. The experimental design consisted of 30 plastic aquaria with six treatment combinations (n=5 tanks per treatment). “Mussel treatment” tanks consisted of three *Elliptio complanata* (Lightfoot, 1786) per tank, “shell treatment” consisted of six freshly shucked valves per tank, and control tanks contained lake water only. Two pH conditions were randomly assigned for each treatment to investigate whether pH had an effect on mussel filtration or shell dissolution. Surface water samples were taken from each tank before and after 24h to measure the concentrations of 23 metals in water processed by mussels for 24h compared with controls (ACWWA, 2004). Mussel tissue was also analyzed from a subset of mussels taken from the holding tanks prior to the experiment (control) and from each experimental tank. Mussels were dissected, and tissue was frozen prior to analysis of concentrations of the same suite of metals. Analyses of Variance using General Linear Model were conducted using Minitab, version 21 (Minitab, 2010) to statistically compare changes in metal concentrations between treatments.

The conclusion in Chapter 4 provides an integrative discussion of the lake and tank experimental results and their implications for future work on ecological service valuation and freshwater mussel conservation.

Chapter 2: Freshwater Mussels in Cape Breton Lakes

Introduction

Freshwater mussels (Phylum: Mollusca, Class: Bivalvia, Order: Unionidae), consist of 93 species in 55 genera in Canada, and over 1000 species throughout the world (Metcalfe-Smith *et al*, 2004; Environment and Climate Change Canada, 2017). There have been approximately 30 recorded extinctions of freshwater mussel species in North America since the beginning of the 20^{th} century, and approximately 24 species of Canadian freshwater mussels are considered extirpated, imperilled or critically imperilled, though there are still large gaps in the status assessments of many other species (Lydeard *et al.*, 2004; Haag and Williams, 2014; Ferreira-Rodriguez *et al.*, 2017; Canadian Endangered Species Conservation Council, 2022).

In 2012, a municipal water supply lake in the Cape Breton Regional Municipality (CBRM), Pottle Lake, was confirmed to house a large population of endangered mussels called the Yellow Lampmussel (*Lampsilis cariosa* (Say, 1817)). Yellow Lampmussel is a species of "Special Concern" federally and designated as "vulnerable" within Nova Scotia (Fisheries and Oceans Canada, 2010; Nova Scotia Department of Natural Resources and Renewables, 2022). Pottle Lake is one of only three known populations of the Yellow Lampmussel in Nova Scotia (Fisheries and Oceans Canada, 2010; White, 2016). This discovery was also significant because Pottle Lake (a potable water source) enjoys protections which will potentially safeguard the population of freshwater mussels within the lake. Water supply lakes are designated as Protected Water Areas under the Nova Scotia Environment Act, which prohibits activities in the watershed such as boating, fishing, bathing, burning, use of herbicides and pesticides, waste disposal, harvesting of trees, camping, picnicking, and hunting within the area (Environment Act, SNS 1994; CBRM Water Utility, 2013). Freshwater mussels potentially provide their own benefits to the lake by contributing to its water quality, since these mussels filter suspended particles from the water column and sediment through their feeding behaviours (Phelps, 2005; Haag, 2012).

Since the discovery of Yellow Lampmussel in Pottle lake in 2012 and sampling of lakes throughout the CBRM, researchers have identified the presence of freshwater mussel populations in several other water CBRM lakes (White 2015; White, 2016).

There have been 10 species of freshwater mussels documented in Nova Scotia; with six species occurring in 22 lakes, rivers and streams in Cape Breton County (White, 2015; White, 2016; Davis, 2017). These species are *Elliptio complanata* (Lightfoot, 1796), *L. cariosa, Utterbackiana implicata* (Say, 1829), *Pyganodon cataracta* (Say, 1817), *Leptodea ochracea* (Say, 1817), and riverine species *Margaritifera margaritifera* (Linnaeus, 1758). In Pottle Lake, the most abundant species (83%) is *E. complanata*, followed by *L. cariosa* (9.5%), *Utterbackiana implicata* (5.7%), and *Leptodea ochracea* (Say, 1817) (White, 2016). *L. ochracea* is listed as “Near Threatened”, while *E. complanata, U. implicata,* and *P. cataracta* are listed as “Least Concern” (Cummings and Cordeiro, 2011; Cummings and Cordeiro, 2012a; Cummings and Cordeiro, 2012b; Bogan *et al.*, 2017; Moorkens *et al.*, 2017).

Because of these discoveries, the CBRM Water Utility expressed interest in gaining insight into the role that freshwater mussels may play in lake water quality prior to its entry into water treatment facilities. Towards this aim, my research objectives focus on carrying out a case study characterizing the nature of the potential ecosystem services provided by freshwater mussels within CBRM surface water supply lakes in relation to their ability to improve source water quality and reduce the need for extensive water treatment.

Ecological services

Researchers and policy makers appreciate that the benefits of natural systems have measurable impacts on human economies and well-being (Alcamo *et al.,* 2003), and there are now several accepted theoretical frameworks which allow for estimation and quantification of the monetary benefit of the presence of the resources, organisms, populations, and ecological interactions within a given environment (Alcamo *et al.,* 2003; TEEB, 2010; Saarikoski *et al.*, 2016; Meraj *et al.*, 2021). These inquiries and estimates are often intended to contribute to policy decisions, management and conservation of natural assets, as well as decrease service delivery costs, resulting in a system which prioritizes sustainability, investment and longevity of human industry (Alcamo *et al.,* 2003).

The modern concept of "ecosystem services" arose in the 1970s, as conservation of natural areas came more into the public consciousness (Westman, 1977; Gómez-Baggethun *et al.*, 2009). The Millennium Ecosystem Assessment (Alcamo *et al.,* 2003) brought ecosystem services and their valuation into the mainstream, attracting attention of policy makers in North America. Since then, several conceptual frameworks about ecological services have been adopted by regulatory bodies around the world (TEEB, 2010; Haines-Young and Potschin-Young, 2018).

Population growth and anthropogenic climate change are increasing the rate of resource depletion, habitat destruction, and biodiversity loss on Earth (Altieri & Gedan, 2014; Cavicchioli *et al.*, 2019; Gonçalves-Souza *et al.*, 2020; Cowie *et al.*, 2022). Hardin's (1968) ecological theory of the "tragedy of the commons" proposes that intervention from government is necessary to prevent the exploitation of the natural world. Ecological service theory is intended to influence policy makers and economists to use evidence-based concepts to inform decisions about anthropogenic activities which may impact the integrity of a natural ecosystem (Daly & Farley, 2011). Incorporating ecological services into our economic system is intended to enable policy makers to conceptualize the value of complex processes within an ecosystem without necessarily understanding the ecological background, and it can provide support for policies that protect or enhance ecosystems by demonstrating their economic impact (Daly & Farley, 2011).

The United Nations has adopted the CICES (Common International Classification of Ecosystem Services; Haines-Young & Potschin-Young, 2018) as a common framework by which to classify the different types of ecological services, and this is the primary framework which I will be using for this chapter. The four main categories in this framework are Provisioning, Cultural, Regulating and Supporting. My focus in this chapter will primarily be the latter two categories.

Regulating Services

Regulating and supporting services comprise the main focus of this study, as I attempt to frame the most easily quantifiable services provided by freshwater mussels within the context of the CBRM Water Utility. Regulating services relate to the mediation and control of waste, toxins, air and water flows, as well as the maintenance of environmental parameters (Haines-Young and Potschin, 2018).

The obvious contribution made by freshwater mussels in the context of regulating services relates to improving water clarity and quality. In order to feed on phytoplankton and other microscopic organisms, freshwater mussels actively filter 0.5 - 1L/h of water across their gills' mucous membranes, entrapping suspended particles

(Vaughn, 2008). These particles are transported via ciliary action to a sophisticated sorting organ, where fleshy palps selectively direct particles to the stomach or excurrent siphon based on size and composition - ultimately being digested and excreted or encased in mucous and expelled as pseudofeces, respectively (Haag, 2012). Therefore, the filtration activity of freshwater mussels is responsible for reducing turbidity in the water column by removing silt and inorganic particles as well as plankton and bacteria. Research has also shown that some species of mussels may even remove cyanobacteria from the water column, potentially mitigating harmful algal blooms (HABs)(Waajen *et al.*, 2016). This action is particularly important as cyanobacterial HABs are predicted to increase as climate change progresses over the next several decades (Scholes, 2016; Chapra *et al.*, 2017; Gobler, 2020). The Federation of Canadian Municipalities has created the Municipalities for Climate Innovation Program (MCIP), which has identified the management of natural assets as a strategy for increasing climate resilience (Federation of Canadian Municipalities, 2023). Freshwater mussel populations could be an important natural asset in management of water resources, both for human consumption and ecological integrity.

Other research has shown that freshwater mussels can mitigate the effects of eutrophication and runoff (Waajen *et al.*, 2016; Chowdhury *et al.*, 2016), reduce pharmaceuticals in water (Faust *et al.*, 2009; Ismail *et al.*, 2014; Othman *et al.*, 2015), and even reduce concentrations of pathogenic bacteria such as *E. coli* (Raikow and Hamilton, 2001; Haag, 2012; Ismail *et al.*, 2015).

Supporting services

Supporting services provided by freshwater mussels can be more difficult to quantify. These are services that influence local ecosystems, often indirectly, in a way which affects human life. Research has shown that freshwater mussels can influence micro-currents near the interface between sediment and the water column which can provide habitat for small organisms such as invertebrates and fish (Strayer *et al.*, 1994; Beckett *et al.*, 1996; Haag, 2012). By altering erosion rates and currents in bodies of water, mussels can change the patterns of sediment settlement and energy regimes of a stream or river (Allen and Vaughn, 2011). There may even be ramifications for erosion and river migration patterns, downstream effects, or the recruitment of conspecifics or other organisms (Beckett *et al.*, 1996). Over time, these effects can gradually build to significant changes in the environment, which, along with their filtration and clarifying action in the water column, is why freshwater mussels are often framed as ecosystem engineers or keystone species (Geist, 2010; Darrigran and Damborenea, 2011; Chowdhury *et al.*, 2016; Bolotov *et al.*, 2018; Emery-Butcher *et al.*, 2020).

Freshwater mussels have a hard, calcified shell, which itself can act as a substrate for various epibionts (organisms which live on other organisms), which themselves may influence the environment around them (Gutierrez *et al.*, 2003; Haag, 2012; Vaughn, 2018). In addition to clarifying the water column, the removal of particles and subsequent light penetration alters the assemblage of plankton and macroscopic primary producers in an aquatic system (Chowdhury *et al.*, 2016; Vaughn, 2018). This in turn will affect the food web and the associated trophic interactions in and near the waters in which they live.

Freshwater mussels have long been used by humans as bioindicators of heavy metal contamination due to their ability to bioaccumulate these impurities (Tynan *et al.*, 2005; Sohail *et al.*, 2016; Xu *et al.*, 2022). Mussel shells represent a long-term storage for impurities, isotopes and, much like tree growth rings, their growth patterns can provide a record of environmental conditions during mussel development, from prehistoric to modern times (Blazejowski *et al.*, 2013; Fritts *et al.*, 2017). Mussels have been used as bioindicators of fluctuations in concentrations of metals such as Silver, Arsenic, Cadmium, Cobalt, Copper, Chromium, Nickel, Lead, Selenium and Zinc (*eg.*: Jamil *et al.*, 1999), along with larger scale pollution events of contaminants like mercury (Brown *et al.*, 2005). Mussel soft tissues can be used as biomonitors for shorter term fluctuations, and their propensity to bioaccumulate certain contaminants (such as pharmaceuticals) in soft tissues can provide information about local and upstream conditions (Du *et al.*, 2014; Vaughn, 2018). Tissue sampling typically requires destruction of mussels (*eg.*: Jamil *et al.*, 1999), and therefore tissues of imperiled species are not suitable for use as bioindicators. Due to the sensitivity of juvenile mussels to water quality parameters such as nitrogen loading, the presence and density of juveniles can also be used to qualitatively assess the condition of a water body (Newton & Bartsch, 2007).

Conservation and research

Declines in freshwater mussel populations have been caused by a number of factors, including habitat degradation and loss, damming and other flow changes in water bodies, declines in host fish populations, over-harvesting of mussels and reductions in water quality due to pollution, eutrophication or siltation (Bogan, 1993; Cosgrove *et al*,. 2016). Most research regarding freshwater mussel population declines which focus on water quality demonstrate that poor water quality contributes to mussel declines (with juvenile sensitivity to toxins likely a major factor), but there is little research available whether over-harvesting of mussels decreases the water quality of their habitats (e.g.: Nalepa *et al.*, 1991; Bogan, 1993; Beasley, 2001; Cosgrove *et al.*, 2016).

My study examined correlations between mussel presence and absence in municipal lakes in Cape Breton County and water quality with a focus on turbidity and metal concentrations in lake water.

Methods

Study site description

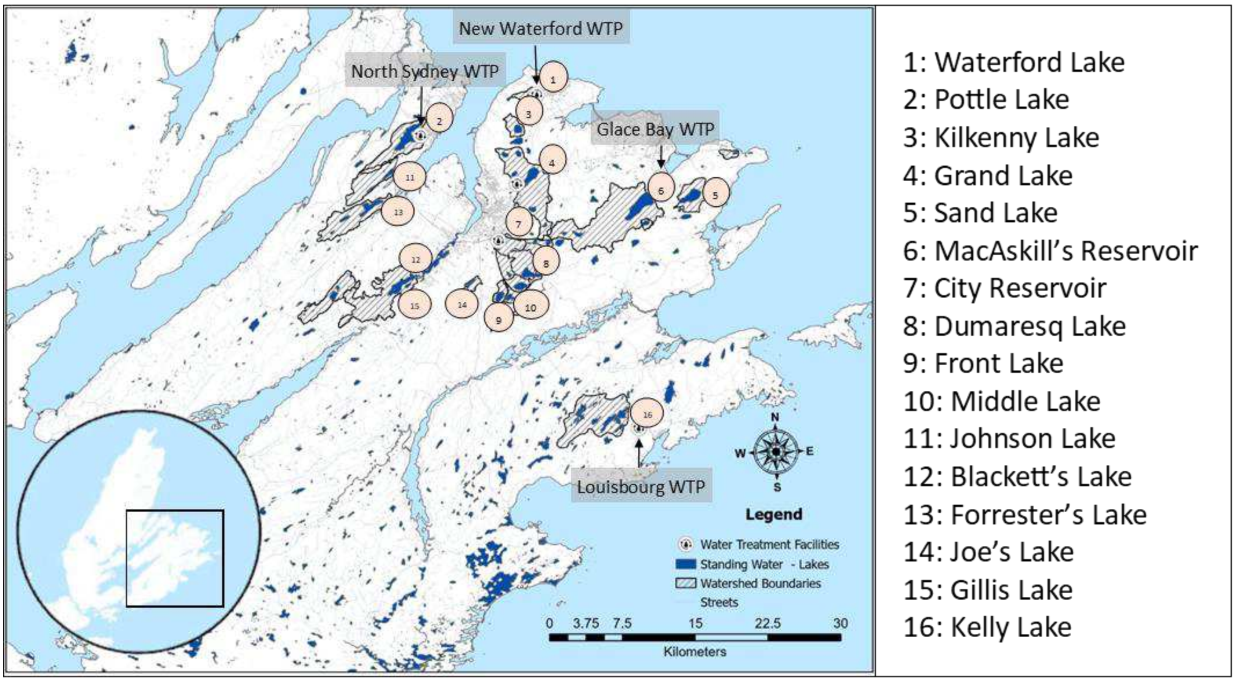

Figure 4. Map showing lakes and water treatment plants in Cape Breton Regional Municipality, N.S.

Sampling in lakes within the Cape Breton Regional Municipality, Nova Scotia, was carried out in order to assess the potential ecological services provided by mussels in relation to water quality within surface potable water supplies. Sixteen lakes were initially selected for analysis, seven containing mussels and nine without (Figure 4; Table 1). Three lakes were removed from the analysis (Kilkenny Lake, Kelly Lake and Forrester's Lake) due to incomplete and/or missing data.

Table 1. Physical properties of selected lakes in Cape Breton Regional Municipality, N.S. Data from NS Lake Mapping tool (Service Nova Scotia, 2023), Data Locator (Province of Nova Scotia, 2023) and Generate Watershed tool from Global Mapper (v21.0; Blue Marble Geographics, 2023). Lakes containing freshwater mussels are italicised.

Lake	**Coordinates (decimal degrees)**	**Surface Area (ha)**	**Watershed area (m^2)**	**Volume (m^3)**	**Mean depth (m)**	**Max depth (m)**
Blackett's Lake	46.066685, -60.311311	172	21 648 194	16 400 000	9.5	30
City Reservoir	46.107061, -60.170160	37	24 082 958	860 000	3	7
Dumaresq Lake	46.074331, -60.138574	64	24 082 958	1 326 870	2.1	4
Front Lake	46.053959, -60.176424	67	2 369 769	1 255 200	1.9	4
Gillis Lake	46.059969, -60.397558	12	9 341 923	268 390	2.3	5
Grand Lake	46.165772, -60.127576	151	24 239 237	2 419 205	1.75	3
Joe's Lake	46.067269, -60.215454	33	1 686 146	414 900	1.2	4
Johnson Lake	46.171725, -60.317649	32	9 220 663	1 396 500	4.4	10
MacAskill's Reservoir	46.137873, -59.980331	329	37 628 867	16 000 000		15
Middle Lake	46.062278, -60.148339	74	24 082 958			
Pottle Lake	46.205268, -60.291347	283	11 849 474	9 800 000		
Sand Lake	46.139151, -59.915631	172	7 429 634	1 950 000	1.5	3
Waterford Lake	46.239235, -60.124404	49	2 466 538			

Surface area of the lakes included in this study ranged from 12ha (Gillis Lake) to 329ha (MacAskill's Reservoir). The next largest lake by surface area was Pottle Lake, followed by Blackett's Lake, Sand Lake and Grand Lake (Table 1). The smallest lakes, in order of increasing surface area, were Gillis Lake, Johnson Lake, Joe's Lake, City Reservoir, Waterford Lake, Kilkenny Lake, Dumaresq Lake, Front Lake and Middle Lake; Table 1). MacAskill's Reservoir also had the largest watershed ($37.6km^2$), followed by Grand Lake ($24.2km^2$), and then Middle Lake, Dumaresq Lake and City Reservoir, which encompass the Zone of Contribution for the Middle Lake Wellfield ($24.1km^2$). Public data was not available for all lakes, but the data available for volume show a range of $414900m^3$ (Gillis Lake) to 16.4 million m^3 (Blacketts Lake; Table 1).

The watersheds of most of the lakes in this study are primarily comprised of land zoned as Rural CBRM (Table 2). Six lakes have portions designated residential (rural or urban), and five allow some industrial activity (Table 2).

Table 2. Land use in watersheds for lakes used in comparative observational study. Lakes containing freshwater mussels are italicised.

	Land-use Zones in Watershed					
Lake	Public Water Supply Watershed Zone*	Rural CBRM Zone**	Rural residential ***	Urban residential ****	Industrial *****	Outside of CBRM Jurisdiction
Grand Lake	X	X		X	X	
Gillis Lake		X		X	X	
Pottle Lake	X	X	X	X	X	
Joes Lake		X				X
Dumaresq Lake	X	X				
Front Lake	X	X				
Johnson Lake	X	X				
MacAskill's Reservoir	X	X		X	X	
Middle Lake	X	X				
City Reservoir	X	X				
Sand Lake		X				
Blacketts Lake			X	X	X	
Waterford Lake	X	X		X		

*Public Water Supply Zone prohibits fishing, bathing, boating, hunting, waste disposal, burning, camping, picnicking, and the use of biocides.
**The Rural CBRM (RCB) Zone permits all agricultural and fishery uses as well as a wide variety of service and recreational uses. Mobile homes (a.k.a. mini homes), single detached dwellings and two-unit dwellings are permitted.
***Rural residential includes Rural Residential Subdivision Zone and Rural County Estate.

****Urban residential includes Residential Urban C Zone, Residential Urban D Zone, Residential Urban A Mini Home (RUAM) Zone and New Dawn Retirement Village Zone. These include predominantly residential uses including single detached dwellings and two-unit dwellings, mobile homes (a.k.a. mini homes). Apartment buildings are not permitted.

*****Industrial includes the Arterial Business Corridor Zone, Regional Industrial Utility Zone, Rural Gravel Deposit Zone and Utility Generation/Treatment/Disposal Zone. The Arterial Business Corridor (ABC) Zone permits all sales uses as well as a wide variety of other business developments and residential uses. Mobile homes (a.k.a. mini homes) are not permitted.

Lake Analysis

Previous studies have demonstrated that freshwater mussels significantly impact water parameters such as dissolved organic carbon (DOC), turbidity, and algae concentration (Nicholls and Hopkins, 1993; Strayer *et al.*, 1999; Roditi *et al.*, 2000), and others have shown that mussels can accumulate metals in tissues and shells (Zuykov *et al.*, 2013; Jing *et al.*, 2019; Khan *et al.*, 2019; Le *et al*, 2020). However, less has been done to investigate the impact which freshwater mussels have on metals in the water column.

Surface water samples (general chemistry = 250ml bottle, nutrients = 250ml bottle with H_2SO_4, and metals = 120ml bottle with HNO_3; Bureau Veritas, 2023) were taken from each lake in November 2020, October 2021, and July 2022, and sent to a commercial laboratory (Maxxam Analytics / Bureau Veritas, CBRM) for testing.

RCAp-MS analysis was done on all water samples collected from study lakes to measure 47 parameters, including major ions, 23 metals, and other physical-chemical characteristics (ACWWA, 2004). This proprietary method is the recommended protocol used by CBRM Water Utility, and therefore it was selected for use in this research in order to give the ability to compare with data sets previously collected by CBRM, and for use in internal reports in the future (ACWWA, 2004; A. Mazzocca, personal communication, September 2021). The primary method of metal analysis within this protocol is EPA Method 6020B: Inductively Coupled Plasma - Mass Spectrometry (U.S. E.P.A., 2014). General chemistry and nutrient parameters were measured as part of the RCAP-MS Assay. Samples were solubilized or digested prior to analysis according to the manufacturer's instructions, and then analysed using a mass-spectrometer. A radio-frequency inductively coupled plasma torch inside the device heats an aerosolized solution, producing ions, which then are fed into the mass-spectrometer, sorted according to mass:charge ratio and then quantified via channel electron multiplier (U.S. E.P.A., 2014). All analyses included quality control (QC) samples including a method blank, a matrix spike, a laboratory control sample (LCS), and a duplicate sample (U.S. E.P.A., 2014).

Two-way ANOVAs were conducted using Minitab, version 21 (Minitab, 2010) with Lake Type (mussel vs. non-mussel) and date as the independent variables and water parameters as the dependent variable, to determine whether there were correlations between mussel presence and each metal or water chemistry parameter. A p-value <0.05 indicated a significant difference in parameters between main effects (lake type, date). A significant interaction effect ($p<0.05$) indicated that sampling date caused a significant difference between mussel and non mussel lakes over time.

Treatment facilities description

Four water treatment facilities in the CBRM Water Utility were selected for analysis, representing three methods of drinking water treatment (Figure 4). These facilities were selected because they utilize surface water for drinking water sources, and two of the lakes (Pottle and Waterford) contain populations of freshwater mussels, and two (MacAskills and Kelly Lake) do not have mussels (White, 2015; White 2016). Since this water utility consists of only seven treatment facilities, and only the four selected facilities use surface water supplies (as opposed to ground water / well supplies), this analysis allows a snapshot view into the effects of source water quality, treatment process, and costs associated with treatment.

The Glace Bay water treatment facility (source water MacAskill's Reservoir) is a flocculation-sedimentation system, while the North Sydney water treatment facility (source water Pottle Lake) uses an ultrafiltration (UF) membrane filtration system (A. Mazzocca, personal communication, November 2021). New Waterford (source water Waterford and Kilkenny Lake) and Louisbourg (source water Kelly Lake) water treatment facilities use dissolved air-floatation systems (A. Mazzocca, personal communication, November 2021).

Glace Bay

The Glace Bay water treatment facility process consists of two pre-treatments, the first is the addition of lime ($Ca(OH)_2$) and potassium permanganate ($KMnO_4$), and the second consists of the addition of CO_2 , polymer, and aluminum sulphate $Al_2(SO_4)_3$ (A. Robinson, personal communication, December 2021). Potassium permanganate is used to oxidize dissolved iron and manganese, which allows these metals to precipitate out of the solution, a process enhanced by increasing pH (in this case, achieved by adding lime) (Welch, 1963; A. Robinson, personal communication, December 2021). Aluminum sulphate is a salt which dissociates in aqueous solution into a cationic form which adsorbs onto negatively charged precipitates (such as those formed in pre-treatment 1), allowing larger conglomerates to form and settle out of the water (Jiang, 2001). This process works most efficiently in low pH, so carbon dioxide is injected into the water to reduce pH via the formation of carbonic acid (Jiang, 2001; A. Robinson, personal communication, December 2021).

The water is pumped from the pre-treatment tanks to a flocculation and sedimentation tank, a 28’ deep well that is gently agitated with mechanical mixers, and the precipitates settle on the bottom of the tank until they are drawn off for waste management (A. Robinson, personal communication, December 2021; D. Macaskill, personal communication, 21 September, 2022).

After flocculation and sedimentation, the water is passed through a three-stage filter composed of sand, gravel and anthracite, and then into a contact tank for chlorination. The contact tank is a concrete underground chamber with baffles throughout which create turbulent flow, allowing chlorine to permeate the entire volume of water and interact with any organic contaminants for long enough to sanitize the water. Prior to distribution, fluoride is added and pH is adjusted if necessary with sodium hydroxide (NaOH).

North Sydney

The water treatment facility in North Sydney consists of a pumping station adjacent to Pottle Lake which collects water in an intake chamber and then raw water is gravity-fed across the street to the main treatment facility (S. MacKenzie, personal communication, September 2022). Upon entry to the facility, it is mixed with water from the backwash process. From this holding tank, it is pumped to the membrane filtration chamber (S. MacKenzie, personal communication, September 2022). The membrane filtration system consists of four "trains" - large, robust banks of filter modules. Each train consists of 96 ultra-filter modules, which in turn consist of a primary membrane and two secondary membranes (S. MacKenzie, personal communication, September 2022). Ultrafiltration systems employ filters with a pore size of 5-20nm, which is sufficient for removing most suspended solids and even some heavy metal ions (ACWWA, 2022; Xiang *et al.*, 2022). After passing through these filters, the filtrate water is pumped into a contact chamber, where chlorine is added (see above). After the water passes through the contact chamber, fluoride is added and pH adjusted as described above, and returns to the pumping station for (S. MacKenzie, personal communication, September 2022).

New Waterford and Louisbourg

The water treatment facilities in Louisbourg and New Waterford have the same general process (T. Janega, personal communication, September 2022). Water is gravity-fed from source lakes into the facility, where it is mixed with sodium hydroxide to increase pH, and then poly-aluminum chloride (PAC; $Al_2Cl(OH)_5$) is added as a coagulant (T. Janega and H. LeBlanc, personal communication, September 2022). PAC coagulates suspended particles in a similar fashion to aluminum permanganate, as described above, by attracting negatively charged ions and adsorbing them (Yang *et al.*, 2010). The water is passed into a series of mechanical mixing tanks, each with slower agitation to allow larger flocculant particles to form (T. Janega and H. LeBlanc, personal communication, September 2022). Oxygen is added to water in a saturation tank and then mixed into the flocculated solution, causing the coagulated particles to float to the surface, where it is skimmed by an automated mechanical skimmer into a sludge drain for (T. Janega and H. LeBlanc, personal communication, September 2022).

The remaining water is drained from the bottom of the tanks, passed through a filter of sand, gravel and anthracite, and then into the contact tank for chlorine mixing, then pH adjustment and fluoride addition (New Waterford only), and finally into distribution (T. Janega and H. LeBlanc, personal communication, September 2022).

<u>Water Treatment analysis</u>

An RCAP-MS assay is completed for each water treatment facility on a quarterly basis to ensure water quality (D. MacAskill, personal communication, 21 September, 2022). Data from these reports for each of the four treatment plants was compared, and an average calculated for those with mussel (n=2) and non-mussel (n=2) source lakes. Results were compared with guidance values for metals in Canadian drinking water (Table 3), and percent change from raw water to treated water was calculated for each metal based on the formula:

$$\% \text{ Change} = ((\text{Treated}-\text{Raw})/|\text{absolute difference between raw and treated}|)*100.$$

The annual costs for water treatment chemicals (excepting chlorine and fluoride) and equipment maintenance for each treatment plant for the year 2021 was divided by the estimated number of imperial gallons (igal, 4.55m^3) per year (average daily volume * 365), and then multiplied by 10 000 gallons to determine the cost per 10 000 igal.

Table 3. Guidance values for metals in Canadian drinking water.

Metal	**MAC (mg/L)**	**OGV (mg/L)**	**AOV (mg/L)**	**Reference:**
Aluminum	2.9	0.1	N/A	R1
Barium	2	N/A	N/A	R1
Cadmium	0.007	N/A	N/A	R1
Copper	2	N/A	1	R1
Iron	N/A	N/A	≤ 0.3	R1
Lead	0.005	N/A	N/A	R1
Magnesium	N/A	N/A	N/A	R1
Calcium	N/A	N/A	N/A	R1
Manganese	0.12	N/A	≤ 0.02	R1
Sodium	N/A	N/A	≤ 200	R1
Strontium	7	N/A	N/A	R1
Zinc	N/A	N/A	≤ 5.0	R1
Boron	5	N/A	N/A	R1
Cobalt	N/A	N/A	N/A	R2
Nickel	0.1	N/A	N/A	R3
Phosphorus	N/A	N/A	N/A	R4
Selenium	0.05	N/A	N/A	R1
Silver	N/A	N/A	N/A	R1
Titanium	N/A	N/A	N/A	R5

MAC: Maximum acceptable concentration; OGV: Operational guidance value; AOV: Aesthetic objective value.
References: R1: Health Canada, (2022), "Guidelines for Canadian Drinking Water Quality".
R2: BC Government, (2004), "Ambient Water Quality Guidelines for Cobalt"
R3: ATSDR, (2005), "Public Health Statement - Nickel"
R4: Canadian Council of Ministers of the Environment, (2004), "Phosphorus: Canadian Guidance Framework for the Management of Freshwater Systems"
R5: WHO, (1982), "Environmental Health Criteria 24 - Titanium"

Results

Water treatment costs ranged from \$3.11 to \$10.80 per 10 000 igal (Table 5). Chemical and equipment maintenance costs for 2021 were highest for the Glace Bay water treatment facility, and lowest for Louisbourg (Table 5). When grouped by mussel presence in source water, treatment facilities without freshwater mussels spent \$3.44 - \$7.69 more per 10 000 igal than those with mussels (Table 4).

Table 4. Estimated treatment costs (\$CAD) per 1000 imperial gallons (igal; $4.55m^3$) in 2021 for four water treatment facilities in Cape Breton, Nova Scotia, based on average daily flow and total yearly chemical and equipment maintenance costs. Calculations do not include the costs of chlorine, labour or electricity.

Water Treatment Facility	Mussels present?	Chemical costs	Equipment Maintenance costs	Average Daily flow (igal)	Total Cost per 10 000 igal
Pottle Lake	Yes	\$140,000	\$98,500	2,100,000	\$3.11
New Waterford	Yes	\$120,000	\$53,000	1,300,000	\$3.65
Glace Bay	No	\$350,000	\$116,000	1,800,000	\$7.09
Louisbourg	No	\$35,000	\$34,000	175,000	\$10.80

Concentrations of aluminum, barium, cadmium, copper, and manganese were lower in both raw and treated water in treatment plants with mussel lakes as water sources (Table 5). Iron and lead were eliminated from treated water in all cases, but the raw water had lower initial concentrations from mussel than non-mussel sources (Table 5). Calcium, magnesium, potassium, sodium and strontium were higher in raw water from mussel lakes, and all but calcium remained at higher concentrations than those with non-mussel sources after the treatment process (Table 5). Non-mussel sources had higher raw water concentrations of zinc, but treated water from mussel sources had a higher zinc concentration (Table 5).

Table 5. Average concentrations (µg/L±SE) of metals for four water treatment facilities in Cape Breton, Nova Scotia, with (n=2) and without (n=2) freshwater mussels present in source lakes. Calculations based on a single quarterly sample taken in March 2022. Percent change was based on the formula:
% Change = ((Treated−Raw)/|absolute difference between raw and treated|)*100.

	Mussel Lakes			**Non Mussel Lakes**		
Metal Concentration (µg/L±SE)	**Raw**	**Treated**	**% Change**	**Raw**	**Treated**	**% Change**
Aluminum	54 (±20)	13.6 (±4.4)	-74.8%	122 (±28)	14.5 (±0.5)	-88.1%
Barium	8.65 (±4.35)	8.2 (±4.8)	-5.2%	4.65 (±1.55)	4.2 (±1.4)	-9.7%
Cadmium	0.007 (±0.007)	0 (±0)	-100%	0.0195 (±0.003)	0.0065 (±0.007)	-66.7%
Calcium	2450 (±350)	2500 (±500)	2.0%	980 (±220)	8050 (±6950)	-721.4%
Copper	1.85 (±1.25)	2.3 (±1.51)	24.1%	13.77 (±13.23)	2.95 (±1.45)	-78.6%
Iron	36.5 (±36.5)	0 (±0)	-100%	270 (±120)	0 (±0)	-100%
Lead	0 (±0)	0 (±0)	0%	0.3 (±0.3)	0 (±0)	-100%
Magnesium	845 (±25)	820 (±60)	-2.95%	590 (±50)	585 (±75)	-0.8%
Manganese	30 (±16)	10.15 (±1.85)	-66.16	79.5 (±60.5)	13.95 (±7.05)	-82.45%
Phosphorus	0 (±0)	410 (±20)	100%	0 (±0)	375 (±45)	100%
Potassium	335 (±5)	355 (±5)	-5.97%	90 (±90)	235 (±125)	161.11%
Sodium	6550 (±1750)	14000 (±0)	113.74%	4600 (±100)	12000 (±1000)	160.87%
Strontium	11.5 (±0.5)	11.5 (±0.5)	0%	6.85 (±1.05)	9.5 (±3.5)	38.68%
Zinc	2.6 (±2.6)	170 (±0)	6438.46%	7.5 (±7.5)	120 (±10)	1500%

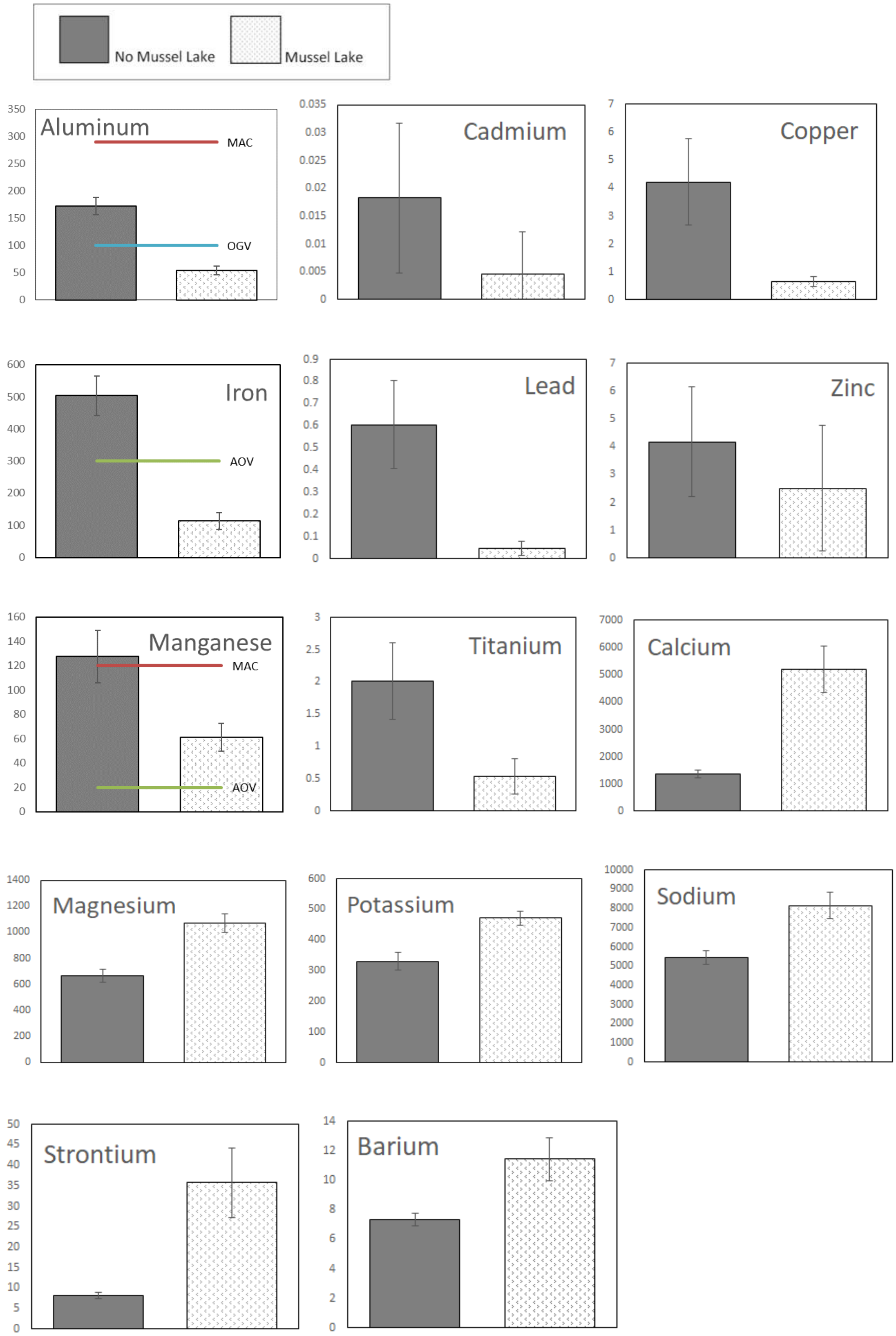

Figure 5. Average (µg/L±SE) concentration of dissolved metals (from lake surface water in mussel (n=8) and non mussel (n=5) lakes in the Cape Breton Regional Municipality ($p<0.05$). Maximum Acceptable Concentration (MAC), Operational Guidance Value (OGV) and Aesthetic Objective Value (AOV) for Canadian drinking water are indicated where they are exceeded by mean concentrations. Data analysed using two-way ANOVA (Minitab v.21).

Lakes with no mussels had an average value for aluminum concentration (172.6μg/L ± 16.3SE) that was higher than the operational guidance value for Canadian drinking water (OGV = 100μg/L) but lower than the maximum acceptable limit (MAC = 2900μg/L; Table 3; Figure 5, Table 3). Mean iron concentration (503μg/L ± 61.3SE; Figure 5) was above aesthetic objective values (AOV = 300μg/L; Table 3) for non-mussel lakes. Average manganese concentration was above maximum acceptable levels (MAC =120 μg/L) for non-mussel lakes (127.5μg/L ± 21.6SE; Fig. X), and above aesthetic objective value (AOV = 20μg/L; Table 3) for mussel lakes (61.3μg/L ± 11.5SE; Table 2; Figure 3). All other values were below the guidelines for Canadian drinking water (Table 3).

For aluminum there was a significant interaction between presence of mussels and sampling date ($F_{2,22}$=3.97, p =0.034). In mussel lakes, aluminum concentration was highest when sampled in July (80μg/L ± 17.5SE), and lowest when sampled in October (29μg/L ± 4.8SE), with November intermediate (54μg/L ± 13.3SE). In non-mussel lakes, aluminum concentrations were lowest in November (145μg/L ± 29.4SE), and concentrations were highest in October (190μg/L ± 27.2SE), July being slightly below October values (183μg/L ± 29.9SE). There were no other significant interactions for metal concentrations between mussel presence and sampling date.

Lakes with freshwater mussels had significantly lower average concentrations of aluminum ($F_{1,22}$=70.24, p <0.001), cadmium ($F_{1,22}$=37.44, p <0.001), copper ($F_{1,22}$=69.75, p <0.001), iron ($F_{1,22}$=34.79, p <0.001), lead ($F_{1,22}$=12.05, p =0.002), manganese ($F_{1,22}$=12.23, p =0.002), titanium ($F_{1,22}$=6.99, p =0.015) and zinc ($F_{1,22}$=4.89, p =0.038; Fig. 2) compared to lakes without freshwater mussels. The greatest difference in concentrations of these metals was lead, with 92% less lead in mussel lakes versus non-mussel lakes, followed by copper (85% less), iron (78% less), cadmium (74% less), titanium (73% less), aluminum (68% less), manganese (52% less), and zinc (40% less).

Concentrations of barium ($F_{1,22}$=56.16, p <0.001), magnesium ($F_{1,22}$=340.09, p <0.001), calcium ($F_{1,22}$=415.79, p <0.001), potassium ($F_{1,22}$=45.04, p <0.001), sodium ($F_{1,22}$=309.31, p <0.001) and strontium ($F_{1,22}$=94.95, p <0.001) were significantly higher in lakes with mussels present (Figure 5). There were no significant interactions between date and mussel presence for these parameters. The greatest difference in these concentrations was strontium, with 77% less in non-mussel lakes, followed by calcium (74% less), magnesium (38% less), barium (36% less), sodium (33% less) and potassium (30% less).

There was a significant interaction between sampling date and mussel presence for turbidity ($F_{2,22}$=4.35, p =0.026). Turbidity was similar for mussel (2.8NTU ± 0.56) and non mussel lakes (2.8NTU ± 0.54) in July samples, however the turbidity was much higher in non mussel lakes in October (3.7NTU ± 1.07 non mussel; 1.11 ± 0.21 mussel) and November (3.1 NTU ± 0.75 non mussel; 2.2NTU ± 0.3). Lakes with freshwater mussels had significantly less turbidity ($F_{1,22}$=10.49, p =0.004), colour ($F_{1,22}$=148.70, p <0.001), and organic carbon ($F_{1,22}$=59.14, p <0.001) than lakes without mussels (Figure 5).

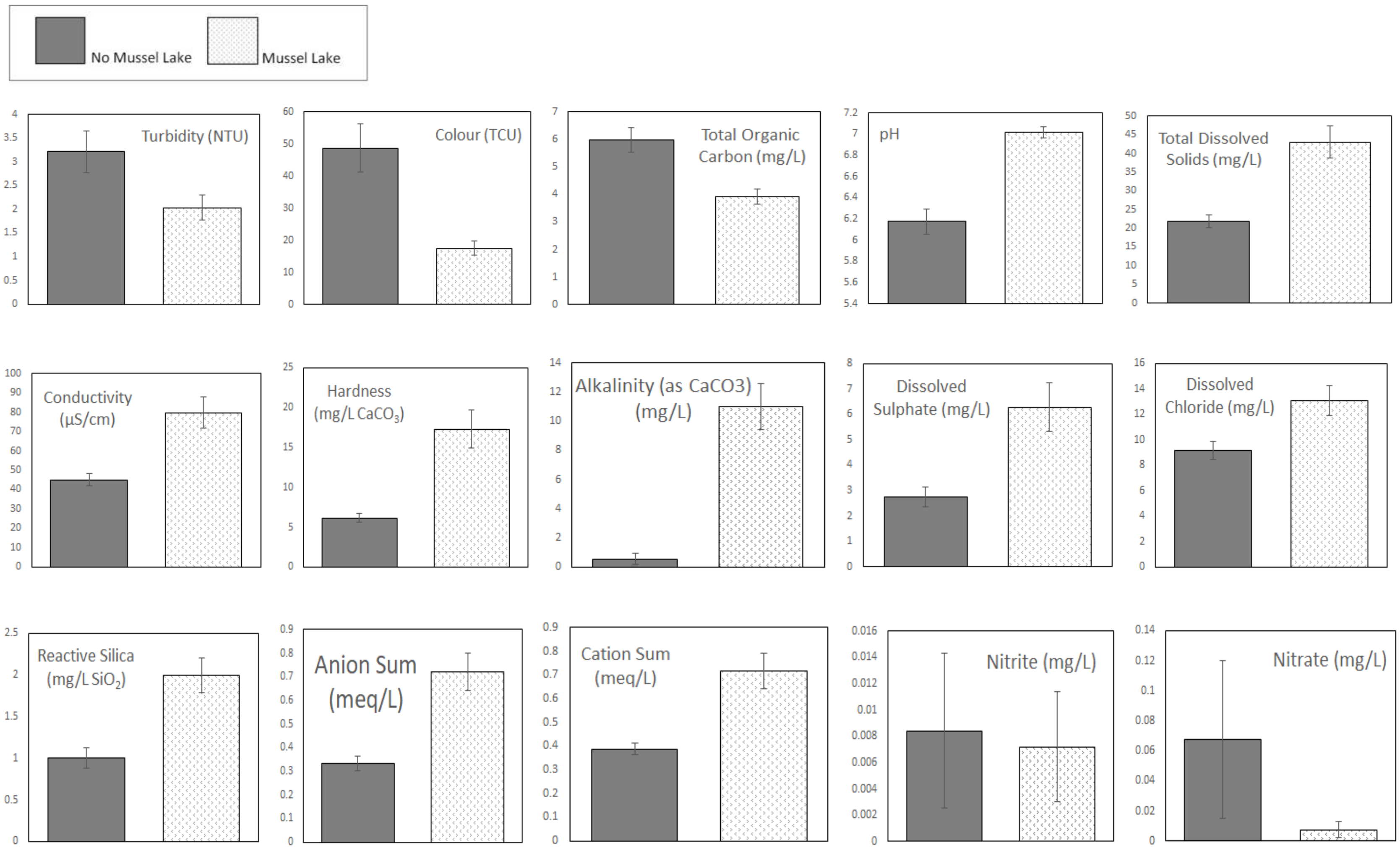

Figure 6. Average (±SE) water parameters from lake surface water in mussel (n=8) and non mussel (n=5) lakes in the Cape Breton Regional Municipality ($p<0.05$). Data analyzed using two-way ANOVA (Minitab v.20).

There was a significant interaction between sampling date and mussel presence for alkalinity ($F_{2,22}$=4.58, p =0.022) and reactive silica ($F_{2,22}$=9.88, p =0.001). Alkalinity in non-mussel lakes was consistently low (0.6mg/L ± 0.64 July; 1.0mg/L ± 1.02 October; 0mg/L ± 0 November), but mussel lakes had higher alkalinity in October (11.64mg/L ± 2.77) and November (12.5mg/L ± 3.27) than in July (8.9mg/L ± 2.33). Reactive silica was lowest in both mussel (0.9mg/L ± 0.16) and non mussel (0.7mg/L ± 0.22) lakes in July, but the concentration increased more in October and November in mussel lakes (2.35mg/L ± 0.33 October; 2.7mg/L ± 0.23 November) than in non mussel lakes (1.0mg/L ± 0.22 October; 1.3mg/L ± 0.17 November).

Lakes with freshwater mussels had significantly higher pH ($F_{1,22}$=99.70, p <0.001), dissolved solids ($F_{1,22}$=355.80, p <0.001), conductivity ($F_{1,22}$=404.99, p <0.001) hardness (as $CaCO_3$; $F_{1,22}$=1188.88, p <0.001), alkalinity (as $CaCO_3$; $F_{1,22}$=341.36, p <0.001), dissolved sulphate ($F_{1,22}$=93.36, p <0.001), dissolved chloride ($F_{1,22}$=97.06, p <0.001), reactive silica ($F_{1,22}$=61.99, p <0.001), anions ($F_{1,22}$=312.18, p <0.001) and cations ($F_{1,22}$=464.63, p <0.001; Figure 6).

There were no significant differences in concentrations of nitrites ($F_{1,22}$=0.03, p =0.861) or nitrates ($F_{1,22}$=2.32, p =0.142; Figure 6).

Discussion

In carrying out this study, I expected to find some correlational evidence that freshwater mussels carry out ecological "Regulating Services" which help control waste and toxins within the water column of lakes that serve as drinking water sources (e.g. Haines-Young and Potschin-Young, 2018). The results are consistent with this expectation, with mussel lakes showing significantly lower levels of impurities such as turbidity and several metals than mussel lakes.

Suspended particles have been shown to be removed by freshwater mussel filtration; mussels obviously remove algal particles upon which they feed, but they also remove inorganic particles, possibly in order to feed on biofilms (a layer of microorganisms which coat particle surfaces; Strayer *et al.*, 1999; Vaughn and Hakenkamp, 2001; Haag, 2012; Kirsch and Dzialowski, 2012). Even without ingesting inorganic particles, mussel filtration still removes significant amounts of silt and other debris from the water column through the capture and sorting of rejected particles into agglutinated pseudofeces (Haag, 2012). It is likely that some of these impurities are bound to suspended particles in the water column, and therefore suspension feeding by mussels is likely to be driving the initial flux of a number of impurities from the water column into mussel bodies (Gaur *et al.*, 2005; Haag, 2012). Once ingested, particles are either passed through mussel digestion or deposited on the benthos as pseudofeces.

Many studies have demonstrated the change in water quality associated with the introduction of an invasive freshwater zebra mussel, *Dreissena polymorpha* (Pallas, 1771), with water clarity and quality significantly and rapidly increasing upon introduction (Fahnenstiel *et al.*, 1995; Kirsch and Dzialowski, 2012). Howard and Cuffey (2016) showed that a species of Unionid mussel, *Margaritifera falcata* (Gould, 1850), could deposit 12.6-13.8mg/h of material on the benthos per mussel, representing a significant active flux of material from the water to the stream bed due to mussel activity. In other studies, freshwater mussels were shown to continue filtering water even at high silt concentrations, removing up to 35% of suspended particles from the water column (Lummer *et al.*, 2016). Strayer *et al.* (1999) found that the presence of zebra mussels resulted in higher water quality due to their equally efficient removal of silt and algae. Therefore, it is expected that freshwater mussel presence in lakes would be correlated with lower levels of turbidity, which was consistent with the results of this study. Increases in turbidity in non-mussel lakes in October and November may be due to increased runoff and siltation due to increased precipitation. The fact that mussel lakes did not exhibit the same increase, and given the fact that these lakes contain populations (often in the range of millions of individuals) of freshwater mussels, it lends evidence to the efficacy of mussel filtration in reducing turbidity.

Based on the demonstrated ability of mussels to remove particulates from the water column, and research showing that metals in the water column are most often adsorbed to suspended inorganic particles (Gaur *et al.*, 2005), I expected that mussel-containing lakes would have significantly lower concentrations of metals such as copper, cadmium and zinc than those without mussels, and my results were consistent with this expectation. Concentrations of aluminum, iron, lead, manganese and titanium were also significantly lower in lakes with mussels (Figure 5). Hussein *et al.* (2022) demonstrated that housing freshwater mussels with an aquacultured fish species resulted in reduction of lead concentration in the fish. Magni *et al.* (2015) showed that freshwater mussels were able to reduce concentrations of aluminum, iron, manganese, nickel, lead and copper in wastewater over 4h of filtration.

Other than deposition onto the substrate, another fate of suspended metals in the water column may be incorporation and sequestration into mussel tissue. Mussels have been found to bioaccumulate metals and other impurities in all soft tissues and hemolymph (Zuykov *et al.*, 2013; Jing *et al.*, 2019; Khan *et al.*, 2019; Yen Le *et al*, 2020). Studies have also shown that mussels bioaccumulate metals in their shells, which would represent a longer-term storage of these impurities (Zuykov *et al.*, 2013; Zhao *et al.*, 2017). Mussels beds vary in density of individuals, but at high densities relative to water volume, this short- or long-term storage of metals may be large enough to have an impact on the water quality (Chowdhury *et al.*, 2016; Vaughn and Hoellein, 2018). The filtration action of freshwater mussels, along with vertical and horizontal movements of these motile organisms alters the water column and substrate of the aquatic ecosystems in which freshwater mussels live, and research has shown that they play an important role in sediment aeration and nutrient cycling between the substrate and water column (Nalepa *et al.*, 1991; Gutiérrez *et al.*, 2003; Aldridge *et al.*, 2007; Vaughn, 2018). Therefore, while metals may be released back into the ecosystem in the form of feces or pseudofeces, or upon the death of the mussel, they may also be incorporated into the substrate via bioturbation for longer term sequestration or incorporated into the food web (including local terrestrial systems) via mussel predation.

Mussel shells are composed of three layers - the inner two composed of calcium carbonate crystals and protein, and an outer layer (called periostracum), composed of primarily proteins (Haag, 2012). Shell formation typically requires a habitat with relatively high concentrations of calcium, a neutral to alkaline pH, and a high buffering capacity (Carroll and Romanek, 2008; Haag, 2012). These characteristics are consistent with the results of this study, with mussel lakes showing higher concentrations of calcium, magnesium, strontium, barium, zinc and copper, as well as higher pH, hardness and conductivity (Figure 5, Figure 6). The presence of these pre-requisite shell building-blocks in the habitat is likely an explanatory factor for why mussels were able to establish and persist within these lakes, however, within Nova Scotia, *Elliptio complanta* is found in lakes with pH as low as 5.3 (Davis, 2007, Nova Scotia Lake Mapping Tool) which indicates that differences in pH between the mussel and non-mussel lakes within this study cannot be explained by lower pH alone. The presence of magnesium and strontium, chemical analogues to calcium, is also consistent with expectations given mussel presence, as they are readily incorporated into shells (Nyström *et al.* 1996; Carroll and Romanek, 2008; Haag, 2012). Trace elements such as barium, zinc and copper have also been measured in mussel shells (Geeza *et al.*, 2019; Hopper *et al.*, 2021) and otherwise are known to be involved in enzyme production (Bairoch, 2000; Waldron *et al.*, 2009). It is possible that increased levels of calcium observed in my mussel lakes is due partially to shell dissolution with environmental pH fluctuations (Nienhuis *et al.*, 2010), however another possible explanation is that these lakes are naturally higher in calcium, creating a favourable environment for mussel growth. While there are some differences in bedrock geology between lakes in different regions of the Cape Breton Regional Municipality (Keppie, 2000), there is no clear correlation between mussel and non-mussel lakes. Therefore the buffering capacity associated with bedrock geology alone is unlikely to explain these differences. Differences in topography, historical land use, groundwater seepage or previous pollution events may contribute to current water chemistry in lakes. Another potential source of water chemistry differences is land use in the watersheds, however, the majority of the lakes in this study are primarily zoned as rural or rural-residential areas (Table 2), and there is no obvious correlation between other land use types and lake water quality: for example, the lakes which have an industrial component as part of the watershed land use all contain mussels and have low values of turbidity (Table 2; Figure 6).

Historical use of the lakes may have some bearing on the question of why there are differences between lakes. In the case of water supply lakes, there are a few confounding factors to consider. First, certain lakes, such as Middle Lake and City Reservoir were used as water supply lakes prior to the opening of the Sydney

Wellfield, and as such were treated with copper sulphate, a potent algicide (van Hullebusch *et al.*, 2003; Albay *et al.*, 2003; A. Mazzocca, personal communication, November 2021). Recent sediment sampling in Middle Lake has shown that high levels of copper persist in the sediment (Dunnington and Spooner, 2019), and since juvenile mussels are both very sensitive to copper and endobenthic, this may explain why freshwater mussels are not found in this area (Lasee, 1991; Naimo, 1995). Second, there are two human-made lakes included in the study, City Reservoir and MacAskill's Reservoir. City Reservoir would have been treated with the aforementioned algicide (A. Mazzocca, personal communication, November 2021), and in addition, when MacAskill's Reservoir was flooded in 1974, trees were not removed prior to inundation (A. Mazzocca, personal communication, November 2021). Submerged wood and soils can contribute high amounts of dissolved organic compounds such as tannins, as well as significantly affecting the ratios of carbon, phosphorus and nitrogen (Campo and Sancholuz, 1998; St. Louis *et al.*, 2000).

While the results of this study are consistent with the expectations of increased water quality due to mussel activity, it is not possible to conclude definitively that the relationship is entirely or partially causal. Mussel ecology is complex due to their position as habitat engineers, their position in the food web, and the added complexity of phytoplankton and fish host dynamics (Haag, 2012; Chowdhury *et al.*, 2016; Vaughn and Hoellein, 2018). For mussels to become established in a habitat, they must first have access to the water body, which in turn must be favourable to mussel, phytoplankton, and fish host survival (Haag, 2012). Some mussels have specific fish hosts; some are even limited to a single species, while others are more cosmopolitan (Haag, 2012). Adult freshwater mussels are able to withstand exposure to toxic metals such as copper, cadmium, nickel, zinc and mercury (Brooks and Rumsby, 1965), however, juveniles and larvae are much more sensitive to toxins such as copper and cadmium (Lasee, 1991; Naimo, 1995). Other environmental pollutants can affect mussel survival, such as salt (Gillis *et al.*, 2022), high turbidity, and eutrophication (Patzner and Müller, 2001; Yusseppone *et al.*, 2020). High levels of siltation and fine suspended particles have been shown to have a negative effect on mussels in several ways: by blocking light and therefore inhibiting growth of phytoplankton which mussels feed on, inhibiting mussel burrowing by hardening substrate, clogging mussel gills, reducing filtration rate, and causing mortality in juveniles (Henley *et al*, 2000). Several studies (*eg.* Kraak *et al.*,1994; Loayza-Muro and Elías-Letts, 2007; Timpano *et al.*, 2022; Bakshi *et al.*, 2023) have demonstrated that complex interactions between nutrients and metal contaminants can cause mortality in freshwater mussels even when limits for individual contaminants are within accepted safe-level ranges. Therefore, high concentrations of certain contaminants may prevent mussel populations from establishing in certain lakes. This suggests that there is likely a range of concentrations for certain metals where freshwater mussels can survive and improve water quality by removing the contaminants from the water, as long as the threshold for mortality (especially of juveniles) is not exceeded.

While it is not possible to say definitively that mussels are responsible for the entire creation and maintenance of high water quality seen in mussel lakes compared to non-mussel lakes, it does appear that non-mussel lakes are costlier to treat for drinking water (Table 4). Historically, there have been many examples of ecosystem services revealing their value once the organism or mechanism has been interrupted or removed, and costs for humans have increased due to the need for replacement services or ecosystem repair (Westman, 1977; Gómez-Baggethun *et al.*, 2009). In Nova Scotia, altered wetlands must be replaced in a compensatory fashion, with two acres of wetland restoration or creation for every acre that has been altered (Government of Nova Scotia, N.D.). Even with indirect-use ecological services, there are ways to estimate the value of freshwater mussels (Strayer, 2017). If the contribution of freshwater mussel filtration on water quality could be quantified (ie: if reductions in turbidity and other impurities were calculated under certain conditions), it may be possible to begin estimating the value of freshwater mussel presence in a source water lake.

Turbidity is the most commonly studied water quality parameter in relation to water treatment costs, because costly chemical flocculants must be added to water to remove suspended particles, which can otherwise clog infrastructure, reduce aesthetic values, and most importantly, bind to toxins and other impurities (Dearmont *et al.*, 1998; Piper, 2003; Heberling *et al*, 2022). My study consisted of a small number of water treatment plants (n=4) so the data are not robust enough to draw any definitive conclusions about mussel presence and the potential effect of mussel filtration on treatment costs, even though the results are consistent with what would be expected if mussel filtration was influencing water quality. Improvements in source water quality, regardless of causal agents, have been directly linked to cost savings in drinking water treatment (Price and Heberling, 2018). In studies with larger numbers of data points, models have been constructed which place a monetary value on water quality improvements, however, economies of scale must be considered, as demand and flow rate can influence the cost per gallon of water treated (Dearmont *et al.*, 1998; Piper, 2003). Price and Heberling's 2018 meta-analysis calculated that the estimated benefit from a 1% reduction in general source water turbidity could range from an annual cost savings of \$121 to \$13,060 (2015 USD) for the average water treatment facility.

Future research could investigate mussel filtration effects on lake water in situ. Mesocosm experiments could be designed in which mussels are excluded from areas of lakes that otherwise contain mussels and compared with areas of high mussel densities in the same lake, while mussels could be placed in non-mussel lakes and water samples compared with water parameters from other areas of the lake. Reducing water movement between treatments would be necessary, however, placement within the natural environment would ensure abiotic conditions such as temperature and light would remain consistent. Monitoring would be required to ensure there are no significant differences between oxygen and algae concentration in mesocosms. Resulting data would provide information about how much mussels contribute to the water quality of a lake compared to natural processes like spring-feeding, runoff, and other biological activity.

Another area of future research could involve the creation of a bio-filter at one or more water treatment facilities, where mussels are allowed to process raw lake water for a period of time prior to treatment, as demonstrated with zebra mussels by Magni *et al.* (2015). Both water parameters and treatment costs could be measured and compared to water that had not been processed by mussels.

Chapter 3: Freshwater Mussel Filtration Study

Introduction

Freshwater mussels are bivalve molluscs in the order Unionidae, comprising 93 extant species in 55 genera in Canada, and over 1000 worldwide (Metcalfe-Smith *et al*, 2004; Environment and Climate Change Canada, 2017). Throughout North America, there have been approximately 30 recorded extinctions of freshwater mussel species since 1900, and ~65% of extant species are considered endangered or otherwise vulnerable (Haag and Williams, 2013) Approximately 24 species of Canadian freshwater mussels are considered extirpated, imperilled or critically imperilled, though there are still large gaps in the status assessments of many other species (Canadian Endangered Species Conservation Council, 2022).

Historically, freshwater mussels have been sustainably exploited by humans as part of traditional foodways and crafts, but starting in the mid-19th century, certain species targeted for pearl harvest and shells for buttons began to decline rapidly, resulting in the industry largely coming to a close by the 1930s-1950s (Kunz, 1898; Anthony and Downing, 2001; Pritchard, 2001). Freshwater mussels are filter feeders, and their intimate relationship with the water in which they live may help to explain the continued high rate of extinction and decline in this group of organisms since the end of the pearl mussel fishery. Freshwater mussels are sensitive to habitat alteration including changes to water flow, sedimentation and pollution, all of which have increased in most freshwater systems due to human activity (Haag, 2012). The life cycle of freshwater mussels involves an obligate fish host, the gills or fins of which provide transportation for mussel glochidia (larvae) during a vital part of their development (Haag, 2012). While certain mussel species can utilize a variety of fish species as hosts, some are dependent on a single species of fish. Therefore, any anthropogenic pressures which affect the success of their fish hosts will increase the precarity of mussel populations. Invasions by non-native zebra mussels in the family Dreissenidae (*Dreissena polymorpha* (Pallas, 1771)) have also caused decline in native freshwater mussel populations (Schloesser *et al.* 1996; Ricciardi *et al.*, 1998).

In areas with lower human impact and more pristine habitat, there are still large populations of freshwater mussels which are thriving in the present day, especially among freshwater mussels species with more plastic host species and habitat preferences (Haag, 2012; Modesto *et al.*, 2017). An interesting and perhaps unexpected case study of unintentional mussel conservation can be found in lakes which serve as potable water sources for humans; these lakes and their watersheds are protected by law, and often therefore remain in pristine condition (CBRM, 2013). Some water supply lakes are designated as Protected Water Areas under the Nova Scotia Environment Act, which prohibits activities in the watershed such as boating, fishing, bathing, burning, use of herbicides and pesticides, waste disposal, harvesting of trees, camping, picnicking, and hunting within the area (Environment Act, SNS 1994; CBRM Water Utility, 2013). In the Cape Breton Regional Municipality, several source water lakes were found to house substantial populations of freshwater mussels. Pottle Lake, the drinking supply for North Sydney, Nova Scotia and surrounding areas (46.1209, -60.1730) was found to have an abundance of freshwater mussels (White, 2016). This lake has a volume of 9.8 million m^3, surface area of 283ha, and a watershed of 11.8 km^2 (Spooner, 2010; CBRM, 2013). Pottle Lake was surveyed and found to contain four species of mussels, comprising approximately 5.5 million individuals (White, 2016).

Lampsilis cariosa (Say, 1817), the Yellow Lampmussel, was one of the four species found in Pottle Lake (White, 2015). The discovery of *L. cariosa* in this lake was significant because this species has only been found in four waterways in Canada: the Saint John River, New Brunswick, and Pottle, Blackett's and Forrester's Lakes in Cape Breton Island, Nova Scotia (Cosewic, 2014; White, 2016). Yellow Lampmussel was listed as Threatened under the Nova Scotia Endangered Species Act in 2006 and listed as Special Concern under Schedule 1 of the federal Species at Risk Act (S.C. 2002, c. 29) in 2005. In Pottle Lake, the most abundant species (83%) is *Elliptio complanata* (Lightfoot, 1796), followed by *L. cariosa* (9.5%), *Utterbackiana implicata* (Say, 1829)(5.7%), and *Leptodea ochracea* (Say, 1817)(White, 2016). *L. ochracea* is listed as "Near Threatened", while *E. complanata, U. implicata,* and *P. cataracta* are listed as "Least Concern" (Cummings and Cordeiro, 2011; Cummings and Cordeiro, 2012a; Cummings and Cordeiro, 2012b; Bogan *et al.*, 2017; Moorkens *et al.*, 2017).

The Yellow Lampmussel and other mussels in Pottle Lake may also be natural assets, benefiting humans by improving the water quality of the lake. Freshwater mussels are very efficient filter feeders; individual adult mussels are capable of processing ~0.5-1L of water every hour (Vaughn *et al.* 2008). Therefore, the estimated 5.5 million freshwater mussels of Pottle Lake have the capacity to filter between 2.75-5.5 million L/h and could potentially filter the entire lake volume in 74-148 days. The raw water quality of Pottle Lake is very high, exceeding max acceptable levels and even aesthetic objectives for all impurities (CBRM, 2013). This may indicate, therefore, that the relationship between humans and mussels in this case is mutually beneficial to both parties, with freshwater mussels providing ecosystem services that benefit humans, and the regulations and protections which restrict human activity in the lake and watershed contributing to the integrity of a critical refuge for an important and threatened taxon of invertebrates.

Freshwater mussel filtration

Freshwater mussels are known to significantly alter the properties of their habitat by filter feeding, bioturbation of sediments, altering near-bottom water flows and sediment rates, and providing substrate for epibionts (organisms which use other organisms as substrate upon which to live), and can therefore be thought of as ecosystem engineers (Nalepa *et al.*, 1991; Strayer *et al.*, 1994; Gutiérrez *et al.*, 2003; Aldridge *et al.*, 2007; Chowdhury *et al.*, 2016; Vaughn, 2018). Bioturbation is the mixing and movement of sediments, and mussels do this by moving both vertically and horizontally throughout the sediment, using their fleshy foot (Haag, 2012; Vaughn, 2018).

The diet of freshwater mussels includes phytoplankton, detritus, fungal spores, dissolved organic carbon, glucose and NH_3, and a large portion of their diet is often comprised of bacteria - including biofilms which coat detritus and suspended particles in the water column (Vaughn *et al.*, 2008; Roditi *et al.,* 2000). Particles ≤20 µm in the water column, such as silt, algae and even cyanobacteria are likely to be entrapped and removed from the water column by mussel filtration, which have been shown to improve the quality of the water column in terms of turbidity, colour, and removal of potentially harmful heavy metals, which are often bound to silt and other suspended particles (Gaur *et al.*, 2005; Haag, 2012; He *et al.*, 2014; Pejman *et al.*, 2015; Aradpour *et al.*, 2020). Freshwater mussels have also been shown to remove pathogenic viruses such as influenza, bacteria such as *Escherichia coli*, pharmaceuticals, herbicides and other contaminants from water via filtration (Faust *et al.*, 2009; Ismail *et al.*, 2014; Othman *et al.*, 2015).

Because freshwater mussels are long-lived, sessile filter feeders, they have the ability to provide information about the environment they inhabit. Freshwater mussels may incorporate toxins or other indicators of environmental conditions into their shells, which can provide a historical record of conditions in waterways, including climatic events and other changes in water quality (Jamil *et al.*, 1999; Brown *et al.*, 2005; Rocha *et al.*, 2015; Binkowski *et al.*, 2019) Mussel soft tissue can provide indications of the current health of the ecosystem or recent events, not only by incorporation of contaminants but also by physiological indicators such as immune response and gene expression (Kolarevic *et al.*, 2016). Some examples of this research include pharmaceuticals, metals, and agricultural runoff (Cope *et al.*, 2008; Wen *et al.*, 2010; Atkinson *et al.*, 2014; Du *et al.*, 2014).

Various regulatory agencies have put guidelines in place to ensure that heavy metal contamination of drinking water does not put consumers at risk (*i.e.,* Health Canada, 2022; United States Environmental Protection Agency; 2023). Heavy metals can enter drinking water sources in a variety of ways. A meta-analysis by Chowdhury *et al.* (2016) showed that air pollution, soil leaching, industrial effluent, agriculture, and weathering of local geological features can all affect heavy metal concentrations in source water, whether from surface or groundwater. The same meta-analysis examined studies relating to the effects of contaminated drinking water and found that such ingestion of metals such as arsenic, cadmium, chromium, copper, lead, nickel, and zinc were correlated with increased risk of stillbirth and cancer, and had negative effects on the skeletal, respiratory, cardiovascular, integumentary, and urinary systems of the human body.

Research Objectives

The objective of this research project was to investigate the potential role freshwater mussels play in changing metal concentrations in lake water prior to treatment in municipal water treatment facilities for public consumption. Towards this objective, a series of tank studies were undertaken to determine the fate of metals in the water column when exposed to live filtering freshwater mussels and mussel shells. The water column and mussel tissue were sampled for changes in metal concentrations over time. The impact of varying levels of water pH on changes in metal concentrations over time was also investigated in this study in order to better understand how the impact of mussels and mussel filtration on metals may vary across lakes with varying pH levels.

Methods

Study site descriptions

MacAskill's Reservoir

Raw lake water was collected from MacAskill's Reservoir (Figure 7; Cape Breton County, NS, 46.137015, -59.977808) for use in all tank experiments. This human-made lake is the drinking water supply for Glace Bay, NS and surrounding communities and has a volume of approximately 16 million m^3, maximum depth of 17.45m, surface area of 328.9ha and a watershed area of 37.63km^2 (CBRM Water Utility, 2014).

Water from the MacAskill Brook reservoir has been previously characterised by the CBRM Water Utility (2014) as having elevated levels of turbidity, colour, iron, manganese, aluminum, total organic carbon, and alkalinity, which exceed the Guidelines for Canadian Drinking Water Quality (GCDWQ) (Table 3; Dillon, 2004;

Health Canada, 2022). High levels of aluminum may be due to naturally occurring aluminum silicates commonly found in clay particles (Dillon, 2004).

Of all CBRM Water Utility source water lakes, MacAskill's Reservoir (serving the communities of Glace Bay, Dominion, Donkin, Port Morien and surrounding areas), is one of the costliest raw water sources to treat on average (A. Mazzocca, personal communication, September 2021). This lake was created by damming a small stream in the 1970s (MacAskill’s Brook) which has no historical record of freshwater mussel presence, and since no mussels are currently present in the lake, it can be presumed that this water has never been impacted by freshwater mussels filtration (A. Mazzocca, personal communication, November 2021). In addition, the reservoir was completed in 1974 to provide cooling water to a heavy water plant. Because it was intended to be used as cooling water, stumps and other organic material were left in situ in the floodplain when the dam was completed (A. Mazzocca, personal communication, September 2021). This has led to continued challenges with water treatment for this lake, since submerged wood and soils can contribute high amounts of dissolved organic compounds such as tannins, as well as significantly affecting the ratios of carbon, phosphorus and nitrogen (Campo and Sancholuz, 1998; St. Louis *et al.*, 2000).

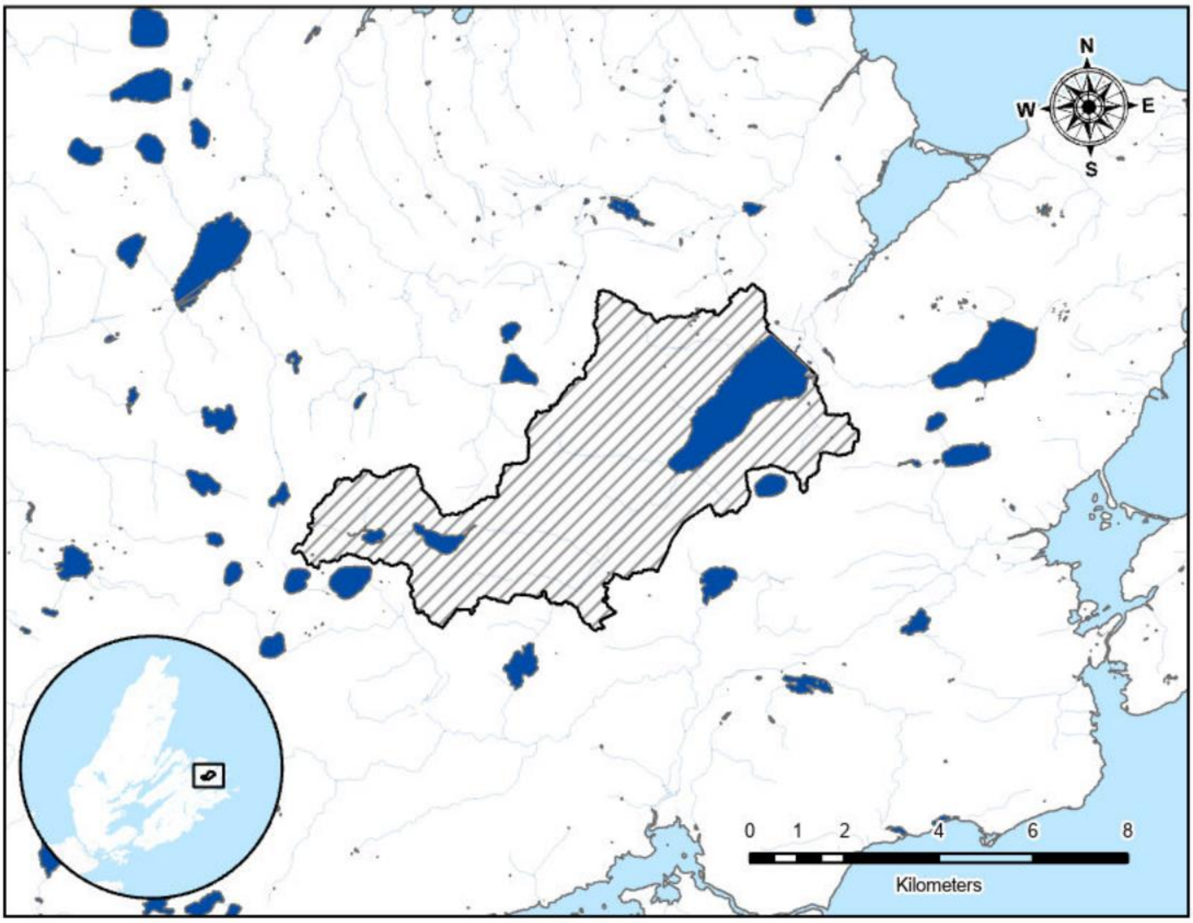

Figure 7. MacAskill's Reservoir and surrounding watershed.

<u>Joe's Lake</u>

All mussels used in my tank experiments were collected from Joe's Lake (Figure 8; Cape Breton County, Nova Scotia, 46.067269, -60.214823) in November 2021. Joe's Lake has a volume of 414900m^3, maximum depth of 4m, surface area of 33.2ha, and a watershed area of 1.69m^2. Joe's Lake has higher pH, turbidity, TDS and conductivity, and higher concentrations of Ba, Ca, Cu, Mg, K, and Sr than MacAskill's Reservoir (Table 6).

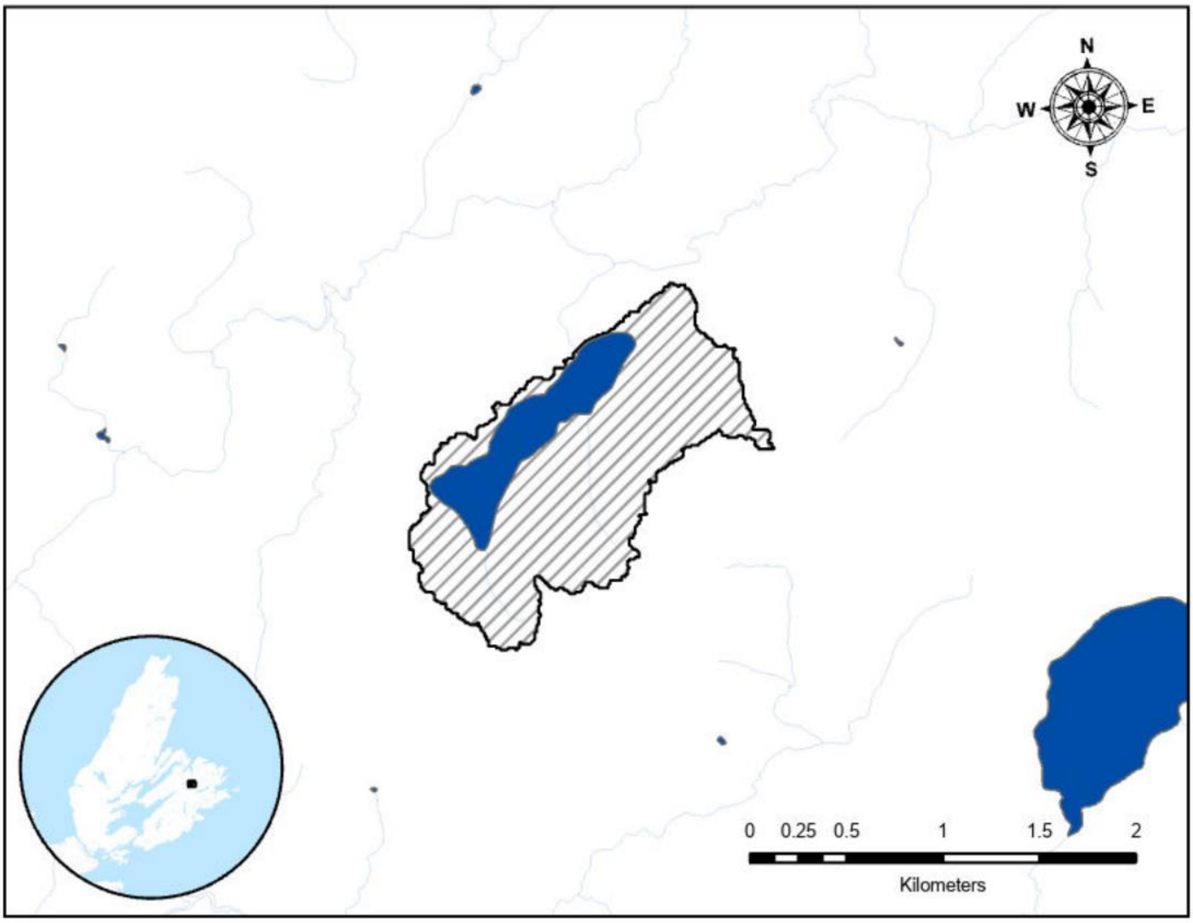

Figure 8. Joe’s Lake and surrounding watershed.

Study species description

The Eastern Elliptio (*Elliptio complanata* (Lightfoot, 1796)) is a Unionid freshwater mussel species found in lacustrine and riverine habitats across northeastern North America from Ontario to Nova Scotia in Canada, and Georgia to Vermont in the U.S.A. (Clarke, 1981; Cummings and Cordeiro, 2011). Individuals can live for >100 years, and reach up to approximately 125mm long, 65mm wide, and 40mm deep, with older individuals appearing dark brown; younger individuals lighter brown to tan (Clarke, 1981).

Table 4. Average concentrations (±SE) of metals (µg/L) and other water chemistry parameters from two lakes in Cape Breton Regional Municipality, sampled in October 2020, November 2021 and July 2022.

	MacAskill's Reservoir	**Joe's Lake**
Aluminum	200.00±15.3	100.33±28.0
Arsenic	0.00	0.00
Barium	6.37±0.7	11.80±1.7
Cadmium	0.01±0.01	0.01±0.01
Calcium	933.33±120.2	2700.00±173.2
Copper	0.17±0.17	0.61±0.3
Iron	770.00±109.7	252.33±135.1
Lead	0.17±0.17	0.18±0.18
Magnesium	693.33±97.01	853.33±66.9
Manganese	153.33±14.5	102.00±59.1
Pottasium	296.67±61.7	513.33±81.9
Sodium	4733.33±611.9	3900.00±346.4
Strontium	8.70±1.01	21.33±2.2
Titanium	2.57±1.5	0.80±0.8
Zinc	0.00	0.00
Turbidity (NTU)	2.37±0.2	3.50±1.2
Colour (TCU)	64.00±11.9	14.70±3.4
Conductivity (µS/cm)	38.67±4.4	43.00±2.9
Calculated TDS (mg/L)	20.00±2.9	25.00±2.1
TOC (mg/L)	6.33±0.5	3.97±0.9
pH	6.07±0.2	6.94±0.05
Anion Sum (meq/L)	0.29±0.04	0.38±0.03
Cation Sum (meq/L)	0.35±0.04	0.40±0.03
Ion Balance (% Diff.)	8.52±2.01	2.55±0.66
Hardness (CaCO3; mg/L)	5.20±0.66	10.33±0.88
Alkalinity (Total as CaCO3; mg/L)	0.00	7.60±0.06
Bicarb. Alkalinity (calc. as CaCO3) (mg/L)	0.00	7.60±0.06
Nitrite (mg/L)	0.02±0.02	0.00
Nitrate (mg/L)	0.00	0.00
Nitrate + Nitrite (mg/L)	0.00	0.00
Dissolved Sulphate (mg/L)	2.90±0.55	3.47±0.32
Dissolved Chloride (mg/L)	8.13±1.16	5.63±0.43
Reactive Silica (SiO2) (mg/L)	1.57±0.18	3.03±0.82

E. complanata is listed globally as a “species of least concern” by The International Union for Conservation of Nature (IUCN) (Cummings and Cordeiro, 2011). *E. complanata* are filter feeders which draw a continuous water supply across their gills through a ciliated incurrent siphon. The mucous coated, ciliated gills conduct particles to the palps, which sort particles based on size and palatability (Riisgard *et al.* 2015). Rejected particles are expelled as pseudofeces through the excurrent siphon, while digestible particles are passed into the digestive system via the mouth, and later expelled through the excurrent siphon as feces following digestion (Haag, 2012).

The feeding mechanism of freshwater mussels involves passing a current of water across mucous covered gills, which capture suspended particles. These particles are moved to palps which direct particles to the digestive system or expel them as mucous-covered pseudofeces (Figure 9; Haag, 2012).

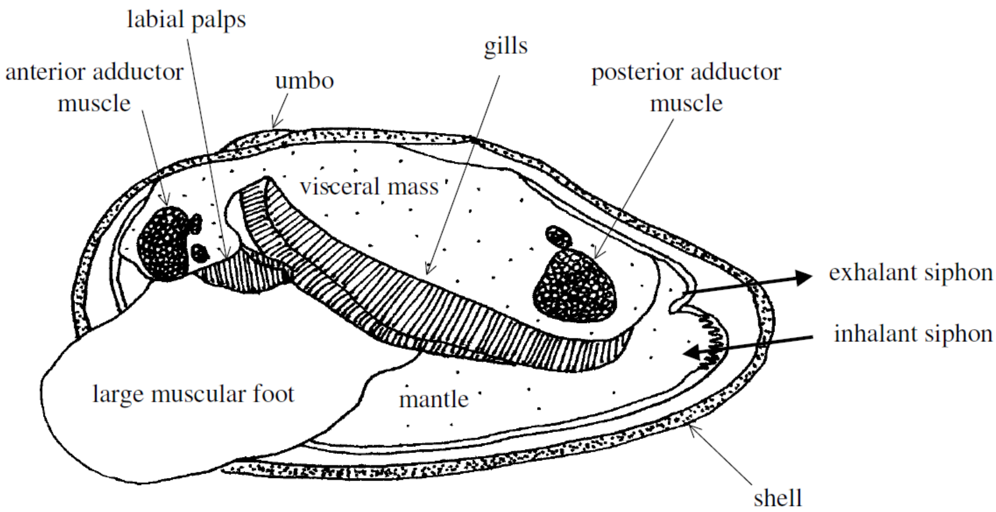

Figure 9. General anatomy of a freshwater mussel. Adapted from Ellis (1978).

Tank experiments

Large *Elliptio complanata* (mean shell length 107.1mm ± 1.4 SE, mean weight 118.4g ± 6.4 SE, n=70) mussels were collected from a shallow, nearshore habitat ~20*3m in area, in Joe's Lake in the Cape Breton Regional Municipality (CBRM) for use in this experiment. Since aging of *E. complanata* cannot be reliably estimated by visual assessment of growth rings, length was used as a proxy for age (Anthony *et al.*, 2001). According to length, these mussels may be estimated to be >40 years of age (Anthony *et al.*, 2001; Schneider, 2006). Prior to tank experiments all mussels were acclimatised in room-temperature, aerated holding tanks containing 5cm deep aquarium gravel substrate and tap water (aged >48h before mussels were added to allow for chlorine evaporation) at room temperature for > 24h prior to experiments, a standard practice for laboratory experiments with suspension feeding bivalves (Spooner and Vaughn, 2008). Mussels were not fed during acclimatisation to allow for digestion and expulsion of gut contents prior to being placed in experimental tanks, as per Spooner and Vaughn (2008). Water from MacAskill's Reservoir, Cape Breton County, NS was used in all experiments (Figure 7).

Previous studies have investigated the effects of mussel filtration on dissolved organic carbon (DOC), turbidity, and algae concentration (Nicholls and Hopkins, 1993; Strayer *et al.*, 1999; Roditi *et al.*, 2000), and others have shown mussels bio-accumulate metals in tissues and shells (Zuykov *et al.*, 2013; Jing *et al.*, 2019; Khan *et al.*, 2019; Le *et al*, 2020). However, there has been less research on mussel impacts on metals in the water column.

Surface water samples (metals = 120ml bottle with HNO_3; Buerau Veritas, 2023) were taken from each tank at 0h and 24h and sent to an accredited commercial laboratory (Maxxam Analytics / Bureau Veritas, CBRM) for testing. Metals analysis was done on all samples collected from the tanks to measure the concentrations of 23 metals (ACWWA, 2004). This proprietary method is the recommended protocol used by CBRM Water Utility, and therefore it was selected for use in this research in order to give the ability to compare with data sets previously collected by CBRM, and for use in internal reports in the future (ACWWA, 2004; A. Mazzocca, personal communication, September 2021). Water and tissue samples were analysed by EPA Method 6020B: Inductively Coupled Plasma - Mass Spectrometry (U.S. E.P.A., 2014). Samples were solubilized or digested prior to analysis according to the manufacturer's instructions, and then analysed using a mass-spectrometer. A radio-frequency inductively coupled plasma torch inside the device heats an aerosolized solution, producing ions, which then get fed into the mass-spectrometer, sorted according to mass:charge ratio and quantified via channel electron multiplier (U.S. E.P.A., 2014). All analyses included quality control (QC) samples including a method blank, a matrix spike, a laboratory control sample (LCS), and a duplicate sample (U.S. E.P.A., 2014).

The experimental design consisted of 30 3L plastic aquaria with six treatment combinations (n=5 tanks per treatment). "Mussel treatment" tanks consisted of three mussels per tank (mean shell length 107.1mm ± 1.4 SE, mean weight 118.4g ± 6.4 SE), "shell treatment" consisted of six freshly shucked valves (shells of three mussels; mean shell length 107.1mm ± 1.4 SE, mean weight 118.4g ± 6.4 SE) per tank, and control tanks contained lake water only. Mussels were scrubbed with a plastic brush and rinsed with tap water prior to shucking and use in the mussel treatment to remove dirt, feces, epibionts and any other material which may interfere with analysis. Two pH conditions were randomly assigned for each treatment to investigate whether pH had an effect on mussel filtration, behaviour or shell dissolution; "low" pH condition was natural lake water pH (6.18±0.03 SE) and “high” was lake water with pH increased to approximately pH 7 prior to the experiment (API pH Up brand sodium carbonate solution; final mean pH was 6.95±0.07 SE). The “shell treatment” and varying pH treatments were included to assess whether the presence of mussel shells in the low pH lake water resulted in changes to metal concentrations regardless of the metabolic and behavioural processes of live mussels. Response variables were the concentration of metals in the water column. This experiment was a fully crossed design, with five replicates for each condition, assigned randomly to tanks (Table 7).

Additional response variables were measured by analysing bodily tissue in addition to tank water at 0h and 24h. Tissue samples were taken from a subsample of mussels at the end of the tank experiment and compared with a subsample of mussels taken from the holding tanks prior to the experiment. One mussel was taken from each experimental tank. Mussels were dissected, and >5g of body tissue was frozen and then homogenised by hand prior to analysis. All mussels were euthanized via freezing following the experiment. Ethics approval for this thesis was granted at the beginning of the research program in 2016, prior to inclusion of bivalves in Dalhousie University’s Animal Care and Ethics Program (Dalhousie University, 2018; Dalhousie University, 2021). As the research took place in laboratory at Cape Breton University, animal care protocols for that institution were followed as best practices, including limiting the number of mussels used in experiments to the minimum, and humane euthanasia following the experiment (Cape Breton University, 2020). All guidelines and permitting required by the Department of Fisheries and Oceans were followed.

Table 5. Details of tank experiments carried out in November 2021.

Treatment	Tank contents	Number of replicate tanks
Mussel, low pH	Live mussels and raw water from McAskill's Lake	5
Mussel, high pH	Live mussels, raw water from McAskill's Lake, and pH up	5
Control, low pH	Raw McAskill's Lake water	5
Control, high pH	Raw water from McAskill's Lake, and pH up	5
Shells, low pH	Empty mussel shells and Raw water from McAskill's Lake.	5
Shells, high pH	Empty mussel shells, raw water from McAskill's Lake, and pH up	5

Data Analysis

Repeated measures analysis of variance (ANOVA) using General Linear Model (GLM) was used to evaluate the effect of mussel filtration on metal concentrations within experimental tanks over a 24-hour period. A significant ($p < 0.05$) interaction between Time (0h, 24h) and Treatment (Mussel, Shell, Control) was interpreted as a significant difference between treatments over time. Data was examined for normality and homogeneity of variance before analysis to assess whether it met the assumptions of parametric statistics, and natural logarithmic (ln) transformation was used to improve heteroscedasticity if necessary. Post-hoc Tukey analysis was used to identify which treatments had pairwise significant differences when ANOVA results showed a p value <0.05. Minitab™ statistical software (version 21) was used for all data analyses.

If metal concentrations were below the threshold of measurement (ie: undetectable) in > 95% of samples, they were excluded from the statistical analysis.

Antimony, arsenic, beryllium, bismuth, boron, molybdenum, nickel, selenium, thallium, tin, uranium, and vanadium were therefore not analysed in tank experiments.

Tissue samples were evaluated using One-Way ANOVA to determine whether there were significant differences between control (mussels taken from holding tanks prior to experiment), and mussels in high pH and low pH conditions. Post-hoc Tukey analysis was used to identify which treatments had pairwise significant differences when ANOVA results showed a p value <0.05. Minitab™ statistical software (version 21).

Results

Overall metals concentration in raw water were ranked in decreasing order: Na>Ca> Fe>Mg>K>Al>Mn>Sr>Ba>Zn>Cu>Ti. Concentrations of Pb, Cr, Co, Cd, Sb, As, Be, Bi, B, Mo, Ni, P, Se, Ag, Tl, Sn, U and V were below the threshold for detection. In the high pH treatment, the order of the major components (>100 μg/L) was similar, though manganese concentration was higher than aluminum (Na, Ca, Fe, Mg, K, Mn, Al, Sr, Ba, Zn, Cu, Pb, Ti, Ag, Cd).

Tank experiments

Aluminum ($F_{2,48}$=10.33, p <0.001) and iron ($F_{2,48}$=14.70, p <0.001) were significantly reduced in mussel tanks after 24h, while barium ($F_{2,48}$=34.79, p <0.001), calcium ($F_{2,48}$=483.07, p <0.001), copper ($F_{2,48}$=23.21, p <0.001), magnesium ($F_{2,48}$=53.37, p <0.001), phosphorus ($F_{2,48}$=46.54, p <0.001), potassium ($F_{2,48}$=10.30, p <0.001), sodium ($F_{2,48}$=12.20, p <0.001), strontium ($F_{2,48}$=1173.59, p <0.001) and zinc ($F_{2,48}$=6.14, p =0.004) all significantly increased in both mussel and shell tanks over 24h (Figure 10). Manganese showed marginally significant reduction ($F_{2,48}$=2.46, p=0.096) over 24h. Cadmium significantly increased ($F_{2,48}$=38.48, p <0.001) in the shell treatment only. Chromium ($F_{2,48}$=1.27, p =0.291), lead ($F_{2,48}$=2.31, p =0.110), silver ($F_{2,48}$=0.10, p =0.909) and titanium ($F_{2,48}$=0.18, p =0.833) did not have a significant change in concentration over time (Figure 10). No significant interactions were observed (treatment*pH, pH*time, treatment*pH*time).

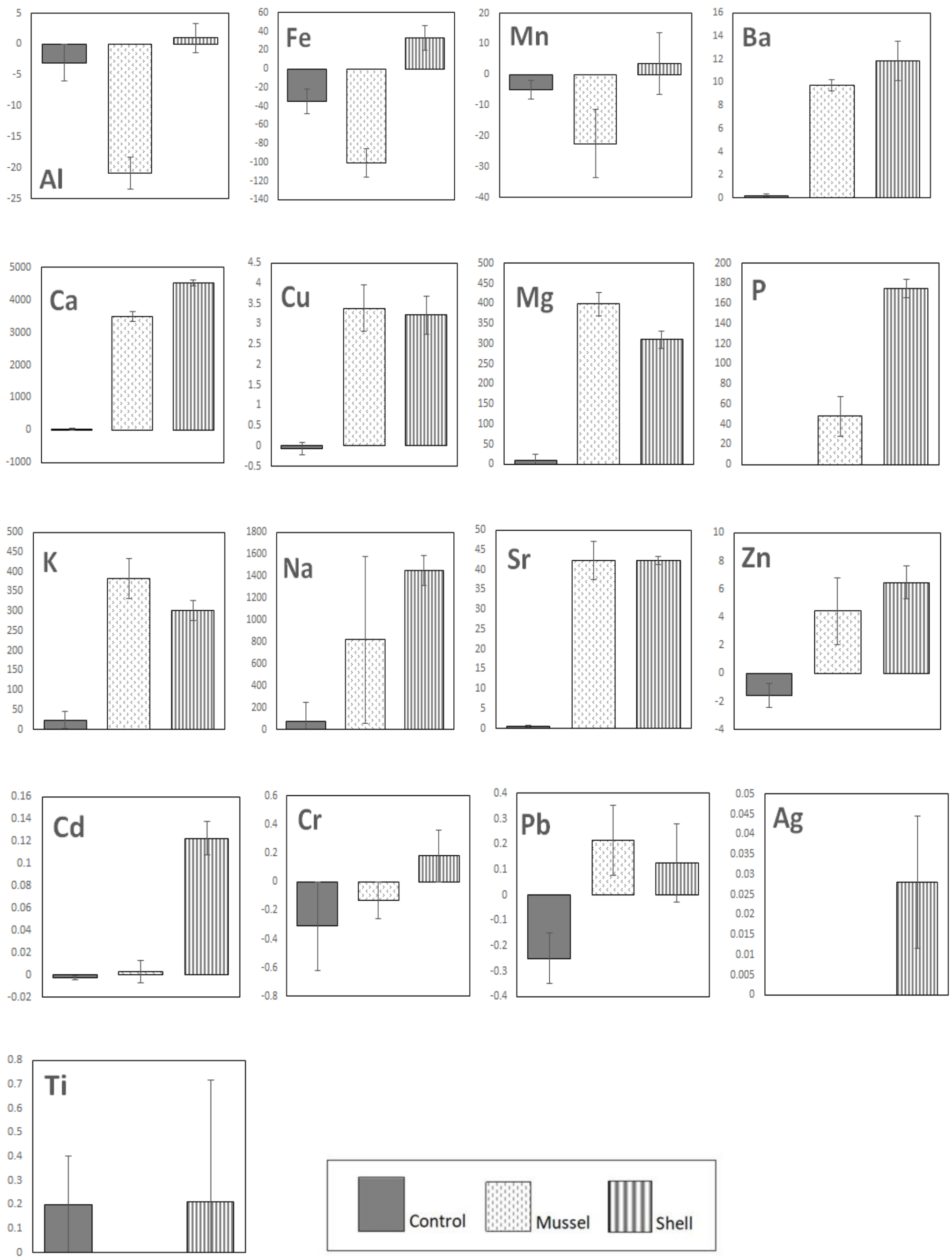

Figure 10. Average (±SE) difference in metal concentrations (μg/L) in water over 24h from tank experiments (Repeated measures ANOVA; p<0.05 significant).

Tissue Analysis

Manganese ($F_{2,15}$=8.48, p =0.003) and zinc ($F_{2,15}$=9.16, p =0.003) had significantly higher concentrations in mussel tissues after 24h filtration of both high and low pH water compared to control mussels (Figure 11). Iron ($F_{2,15}$=3.79, p =0.046) and selenium ($F_{2,15}$=4.52, p =0.029) were shown to have significant differences between control and low pH treatments. Aluminum ($F_{2,15}$=3.29, p =0.057) and copper ($F_{2,15}$=3.36, p =0.062) showed marginally significant differences (0.05>p<0.1) between treatments, but Tukey ad-hoc group comparison showed no significant differences between treatments for copper. Barium ($F_{2,15}$=0.16, p =0.851), cadmium ($F_{2,15}$=1.30, p =0.293), lead ($F_{2,15}$=0.55, p =0.590), silver ($F_{2,15}$=1.61, p =0.232), strontium ($F_{2,15}$=0.95, p =0.408), cobalt ($F_{2,15}$=1.22, p =0.316) and chromium ($F_{2,15}$=1.05, p =0.369) showed no significant differences between treatments (Figure 11). There were no significant differences between treatments for any other metals.

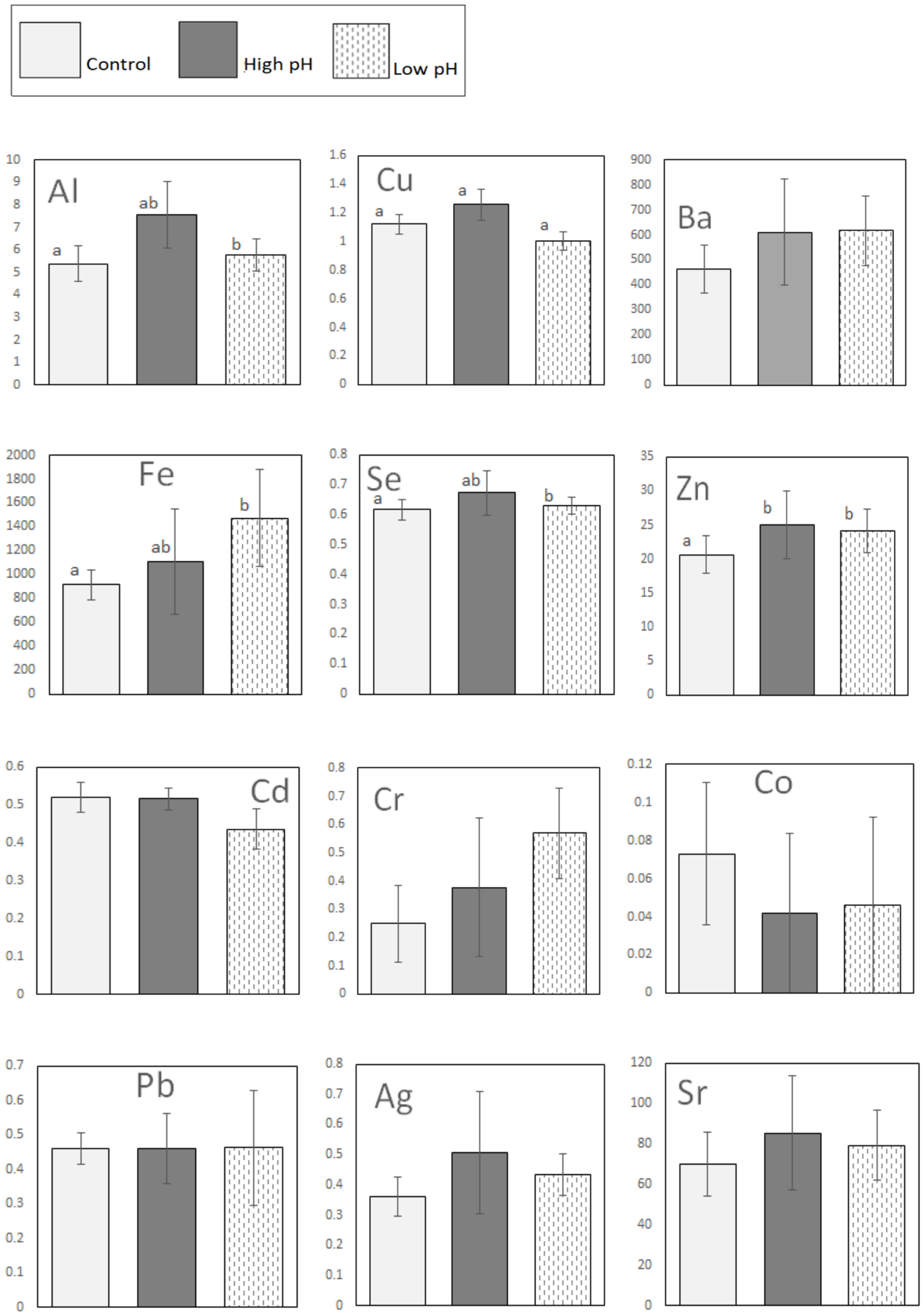

Figure 11. Average metal concentrations (mg/kg±SE) in tissue samples of freshwater mussels in control, high and low treatment conditions. Control mussels were sampled from holding tanks at 0h (n=10), high pH condition mussels were sampled following 24h filtering lake water with pH increased (API pH Up brand sodium carbonate solution; pH = 6.95±0.07 SE; n=5), and low pH condition mussels were sampled following 24h filtering natural lake water (pH = 6.18±0.03 SE; n=5). One-way ANOVA; p<0.05 significant denoted by asterisk. Differing letters above error bars indicate statistical differences according to Tukey post-hoc analysis (Minitab, vers. 21).

Table 6. Concentrations (±SE) of metals from lakes (µg/L), tap water (used in acclimatisation tanks) from Cape Breton University (CBU; µg/L), and freshwater mussel tissue samples (mg/kg). Joe's Lake (n=3 samples; Oct. 2020, Nov. 2021 and July 2022) is the source for the experimental mussels, and MacAskill's Reservoir (n=15 samples, taken at 0h in the experiment) is the source of the water used in the tank experiment. Control mussels (n-10) were randomly selected from holding tanks prior to the experiment, Low pH (n=5) represents mussels which filtered natural lake water from MacAskill's Reservoir (pH = 6.18±0.03 SE) for 24h, and high pH (n=5) represents mussels which filtered lake water from MacAskill's Reservoir that had been adjusted using API pH Up brand sodium carbonate solution (pH = 6.95±0.07 SE) for 24h. Mean metal concentrations in tap water were calculated by an independent contractor (Bureau Veritas, CBRM) from n=4 samples.

	Lakes		**CBU**	**Tissue Samples**		
Metals	Joe's Lake	MacAskill's Reservoir	Holding tanks	Control	Low pH	High pH
pH	6.94 (±0.05)	6.18 (±0.03)	8.0			
Aluminum (Al)	100.33 (±28.03)	133.33 (±19.14)	0	5.38 (±0.79)	5.78 (±0.71)	7.56 (±1.47)
Arsenic (As)	0.00 (± 0.00)	0.00 (±0.00)	0	0.00 (±0.00)	0.25 (±0.15)	0.24 (±0.15)
Barium (Ba)	11.80 (±1.67)	4.80 (±0.20)	200	463.00 (±96.46)	616.00 (±139.59)	610.00 (±212.20)
Cadmium (Cd)	0.01 (±0.01)	0.02 (±0.00)	0	0.52 (±0.04)	0.44 (±0.05)	0.52 (±0.03)
Chromium (Cr)	0.00 (±0.00)	0.29 (±0.22)	0	0.25 (±0.14)	0.57 (±0.16)	0.38 (±0.25)
Cobalt (Co)	0.00 (±0.00)	0.04 (±0.04)	0	0.07 (±0.04)	0.05 (±0.05)	0.04 (±0.04)
Copper (Cu)	0.61 (±0.32)	1.17 (±0.14)	9.3	1.12 (±0.07)	1.00 (±0.06)	1.26 (±0.11)
Iron (Fe)	252.33 (±135.06)	742.00 (±55.03)	0	915.00 (±127.93)	1472.00 (±404.70)	1108.00(±436.14)
Lead (Pb)	0.18 (±0.18)	0.39 (±0.11)	0	0.46 (±0.05)	0.46 (±0.17)	0.46 (±0.10)
Manganese (Mn)	102.00 (±59.07)	130.67 (±4.41)	2	2059.00 (±498.75)	2482.00 (±498.62)	2616.00 (±856.54)
Nickel (Ni)	0.00 (±0.00)	0.00 (±0.00)	0	0.00 (±0.00)	0.00 (±0.00)	0.11 (±0.11)
Selenium (Se)	0.00 (±0.00)	0.00 (±0.00)	0	0.62 (±0.03)	0.63 (±0.03)	0.67 (±0.07)
Silver (Ag)	0.00 (±0.00)	0.00 (±0.00)	0	0.36 (±0.07)	0.43 (±0.07)	0.51 (±0.20)
Strontium (Sr)	21.33	9.31 (±0.14)	650	70.00	79.40 (±17.06)	85.40 (±28.16)
Tin (Sn)	0.00	0.00 (±0.00)	0	0.58	0.00 (±0.00)	0.00 (±0.00)
Uranium (U)	0.00	0.00 (±0.00)	0	0.00	0.00 (±0.00)	0.00 (±0.00)

Discussion

Mussels were found to significantly alter the concentration of metals within my tanks over 24h, though there was considerable variation in the nature of the changes. The reductions of aluminum, manganese and iron indicate that mussels do provide important ecological services within lakes that serve as drinking water supplies. Decreasing concentrations of aluminum, iron and manganese can be beneficial for human consumers and water utilities. High levels of manganese are a concern for infants, causing developmental and neurological problems with memory, attention, and/or motor skills (Department of Health, 2022; Government of Canada, 2023). There is no reason to believe that manganese or iron in drinking water will cause health problems in healthy adults; however, even at lower levels, these impurities can cause discolouration and changes in taste and odour of drinking water, so reductions in these metals would be beneficial to water quality in a municipal water supply (Health Canada, 1978; Husband *et al.*, 2008; IDPH, 2010). The reductions in Aluminum observed does indicate that mussels can contribute to increased water quality that may impact human health. Aluminum in drinking water has been correlated with neurological disorders (Klotz *et al.*, 2017; Gaulthier *et al.*, 2000), and can increase the bioaccumulation of other metals like arsenic, chromium, manganese, nickel, lead, and copper (Health Canada, 2009).

Similar metal reductions in relation to mussel filtration has been observed by other researchers. Magni *et al.* (2015) found that zebra mussels exposed to wastewaters acted as effective biofilters and were able to remove between 20-30% more aluminum, nickel, manganese, iron and lead, and 8% of copper, than were lost by natural sedimentation. Hussein *et al.* (2022) showed that freshwater mussels used in a dual-species aquaculture trial were able to reduce lead concentrations in the primary aquacultured species (a fish). In my tank experiments, a significant reduction in aluminum and iron, and a decrease in manganese were observed (Figure 10), which correspond with expectations based on these previous studies. While nickel was not included in my analysis (due to low concentrations in raw lake water used in the experiments), and lead showed no significant difference between treatments, my tanks had an increase in copper in both shell and mussel tanks.

A major mechanism for removal of impurities from the water column is likely associated with mussel filtration of sediment, algae, and other suspended particles which are transferred from the water column to the sediment via feces or pseudofeces. This process includes aggregation of fine particles into larger and denser deposits held together with mucous (Strayer *et al.*, 1994; Haag, 2012). While this deposition has the potential to be temporary, it is still a stabilising action which then gives time for the biodeposits to be incorporated into the sediment (potentially by mussel bioturbation) or otherwise acted upon by other organisms in the benthos (Strayer, 2014). Reductions in concentrations of iron and manganese may be explained by the fact that these two metals have been shown to be among the most abundant silt-associated particles in lacustrine environments (Pertsemli and Voutsa, 2007). Freshwater mussels, like most filter-feeding bivalves, are also known to incorporate and bioaccumulate trace metals in their tissues and shells (Naimo, 1995; Jamil *et al.*, Sohail *et al.*, 2016). In addition to sequestration, however, there are biochemical reactions taking place within surficial biofilms, tissues and gut (via microbiome activity) of mussels which can change the form, solubility and bioavailability of various metals, which may alter their concentration in the water column (Vaughn and Hoellein, 2018).

While differences in pH did not significantly affect metal concentrations in the water column within my tank experiments, it did have some significant impact on the concentration of metals in mussel tissues after 24 hours of filtering (Figure 11). This may be due to the effect of pH on bioavailability and absorption of certain metals (*e.g.* iron) (Millero *et al.*, 2009; Bates *et al.*, 2021) and may have implications for water treatment and potential future use of mussels as biofilters. The water chemistry of the source lake for the mussels, Joe's Lake, and the water in the holding tanks which the mussels were depurated for >24h prior to the experiment may also have affected the starting (control) tissue metal concentrations. Using water from the source lake in holding tanks prior to experimentation may simplify the results of future studies.

Several metals increased over 24h in my tank trials in both live mussel and shell tanks (barium, calcium, copper, magnesium, phosphorus, potassium, sodium, strontium and zinc; Figure 10). Some of these metals are strongly associated with mussel shells. Mussel shells are composed of three layers - the inner layer nearest to the mantle is the nacreous layer and is composed of calcium carbonate crystals and protein, the intermediate layer is the prismatic layer, also primarily composed of calcium carbonate crystals, but arranged perpendicular to those in the nacreous layer, and the outer layer or periostracum, is composed of primarily proteins, and functions as a protective waterproof layer (Haag, 2012). However, in many cases, the periostracum can be damaged over time and allow acidic water to interact with the exposed calcium carbonate below, leading to shell dissolution (Haag, 2012). Since magnesium and strontium are analogues to calcium with similar chemical properties, they are readily incorporated into shells as well (Nyström *et al.* 1996; Carroll and Romanek, 2008; Haag, 2012). Trace elements such as barium, zinc and copper have also been measured in mussel shells (Geeza *et al.*, 2019; Hopper *et al.*, 2021) and otherwise are known to be important in biological functions and enzyme production (Bairoch, 2000; Waldron *et al.*, 2009). Since the pH of the raw lake water (6.18±0.03 SE) used in my experiment was lower than the pH of the source lake for the mussels (6.94±0.05 SE) and the water in the holding tanks (8.0), it is likely that increases in concentrations of these metals were due to some shell dissolution under acidic conditions (Nienhuis *et al.*, 2010). While there was no statistical difference between high and low pH treatments in the trial, even the high pH treatment (6.95±0.07 SE) was more acidic than the water used in the holding tanks, so shell dissolution is still a likely explanation, and the duration of the trials may have been too short to demonstrate a significant difference in dissolution rates between these treatments.

Phosphorus uptake and release has been observed as a major nutrient flux mediated by live mussels (Atkinson *et al.*, 2014; Strayer, 2014; Vaughn, 2018). Similarly, sodium and potassium ions are vital for cell function (Clausen and Poulsen, 2013). However, this does not explain the increases I observed in shell treatments. It is possible that these were released by live mussels during shucking and remained adhered to the shells even after the light scrubbing and rinsing prior to the experiment, and entered the water column over the 24h trial period. It is also possible that during the preparation of the live and shell treatments, some epibionts remained on the mussel shells (Vaughn and Hoellein, 2018), which may explain some of the differences between these treatments and controls, due to the metabolic actions of these organisms.

High levels of barium ingestion have been correlated with effects on blood pressure and the kidneys (Health Canada, 2019a), however, in general, barium is not found in high enough concentrations in drinking water to cause the adverse health effects (Durham Region Health Department, 2018). Copper deficiency is generally more of a cause for concern than ingesting too much copper, since copper is an essential element in human health (Federal-Provincial-Territorial Committee on Drinking Water, 2018). Healthy individuals are not at risk of adverse health effects from copper in drinking water, however, Wilson's disease can cause sensitivity to copper, resulting in headaches, vomiting, diarrhea, stomach cramps, nausea, liver damage, kidney disease, and blood cell damage (Department of Health, 2023). Strontium behaves similarly to calcium in humans as well as mussels, being readily incorporated into bone matrix, however, high concentrations of strontium can disrupt normal bone development and structure (Health Canada, 2019b; Department of Health, 2019).

Ingestion of zinc in drinking water does not have any adverse health effects except in extremely high concentrations (≥ 675 mg/L), which can cause irritation of the intestines (SCDHEC, n.d.). Zinc ≥40mg/L results in unpleasant taste and cloudy appearance of water, causing it to be unpalatable to many consumers (Health Canada, 1987b).

There are no adverse health effects attributed to the consumption of calcium, magnesium, potassium, phosphorus or sodium in drinking water, unless in extremely high concentrations (Health Canada, 1978; Health Canada, 1987a; Health Canada, 1992; Federal-Provincial-Territorial Committee on Drinking Water, 2008; WHO, 2009; Sengupta, 2013; McVean, 2019; DCCEEW, 2022; EPA, 2023; Region of Waterloo, n.d.).

It is more difficult to explain the increases in cadmium concentration observed in the shell treatment only. Cadmium has been shown to bioaccumulate in mussel tissues, especially the kidneys (Jing *et al.*, 2019). There were no significant differences between treatments in the tissue samples, and tissue concentrations were consistent with low levels in control treatments seen by other researchers (*e.g.* Jing *et al.*, 2019). Pynnönen (1990) observed that cadmium concentrations appear to increase in mussel tissues under neutral to alkaline conditions, which may mean that cadmium can be released or purged from mussels under acidic conditions. However, this would not explain why cadmium concentration increased in shell treatments but did not increase significantly in live mussel treatments in my experiment. Carroll and Romanek (2008) demonstrated higher concentration of metals in the inner nacreous layer of mussel shells, which are protected against exposure to water (and therefore dissolution under acidic conditions) by the mantle in live mussels. It is possible that the freshly shucked shells in my study were releasing higher levels of cadmium into the acidic water in the tanks for this reason.

Persistent exposure to cadmium in low level and persistent doses has been correlated with adverse health effects (Health Canada, 2020; MDH, 2014). Cadmium can decrease bone density and disrupt bone growth, and can build up in the kidneys, eventually causing kidney disease (MDH, 2014).

The toxicity of metals in sediments and water bodies to filter feeding bivalves must also be considered when planning new research or conservation efforts. Economically important and ecologically threatened phyla have been the subject of toxicological studies in relation to their conservation; metals such as cadmium, copper, iron and zinc can be toxic to freshwater mussels, inhibiting growth, reproduction and survival (Naimo, 1995; Wang *et al.*, 2011). Recent research suggests that complex interactions between water quality parameters like pH, hardness, DOC, and certain combinations of dissolved and particle-bound metals can have a compounding effect, increasing toxicity and mussel mortality in some cases (Kraak *et al.*, 1994; Giacomin, 2013).

Conclusions and Future Research

In conclusion, it appears that freshwater mussels have a beneficial impact on water quality by reducing turbidity and concentrations of aluminum, iron, and manganese. Freshwater mussels are long-lived species, with most species' lifespans ranging from 15-20 years, and some exceptional species living for up to 200 years (Haag, 2012). This means that metals and other impurities can potentially be sequestered within mussels' bodies for decades, and longer in the case of shells. There is also the potential for biodeposits, dead mussels and shells to be moved into the benthos, resulting in longer sequestration, or diverted into the food chain. The primary predator of freshwater mussels in Cape Breton is the muskrat (*Ondatra zibethicus* (Linnaeus, 1766)), but wading birds, fishes, and insect larvae have all been identified as mussel predators, and may be a vector for transportation of mussel-accumulated metals throughout the environment (Haag, 2012). Enzymatic action by mussels or their microbiome, or the microbiome of the benthos itself has the potential to change the form and bioavailability of contaminants (Vaughn, 2018; Vaughn and Hoellein, 2018). All of these processes could have positive or negative consequences, and future research is required to investigate the ultimate fates of these impurities.

The potential negative effects of the metals which increased in live mussel and shell trials on health and palatability (barium, strontium, copper, zinc, and cadmium) must be considered prior to future mussel research and potential use in applications such as bio-filtration or translocation. Further research with a wider range of pH could be used to calculate the amount of metals which may be released due to shell dissolution, and acidic lakes may need to be avoided as potential locations for translocation for this reason.

In order to investigate the cumulative effect of mussels on water quality, future research should investigate water parameters in mussel and control tanks for longer durations, to determine the maximum amount of bioaccumulation of metals, and the effects on filtration rate and mortality of mussels. It is possible that the changes in metal concentrations will reach their maximum near the 24h time scale of my experiment, or that new results will arise due to changes in mussel metabolism and behaviour over time. Addition of algae or other types of nutrition to tank trials may also lead to differing results, since mussels' food preference, filtration and sorting behaviour changes depending on type and amount of food present (Allen, 1914; Haag, 2012).

A complete analysis of partitioned mussel tissues, shells, water column and sediment would elucidate the fates of metals, dissolved organic carbon and silt in the presence of mussel filtration. In situ experiments would give an opportunity to investigate these fluxes under natural conditions, and incorporation of microbiome studies could be undertaken for both mussels and sediment. All of this together could help determine the extent and nature of the effects of mussels on water and would provide additional evidence when evaluating the ecological services of freshwater mussels in relation to their impacts on metals.

Chapter 4: General Conclusions

My research has demonstrated that freshwater mussels do perform valuable ecosystem services within municipal water supply lakes. Lake studies have provided evidence that mussel presence is correlated with and may contribute to reductions in harmful or nuisance impurities in surface water sources, such as cadmium, copper, lead, zinc and titanium. The tank study presented evidence of the causal relationship between mussel filtration and improvements in water quality including reductions in metals like aluminum, iron, and manganese as well as reduced turbidity and dissolved organic carbon. One of the metals which increased in tank studies may have adverse health effects (cadmium; MDH, 2014; Health Canada, 2020), however, others are only of concern in large quantities which are unlikely to exist in drinking water sources, such as copper, zinc, and strontium (Durham Region Health Department, 2018; Federal-Provincial-Territorial Committee on Drinking Water, 2018; Health Canada, 2019a; Health Canada, 2019b; Department of Health, 2019; Department of Health, 2023; SCDHEC, n.d.). Most of the metals which increased are not of concern (i.e.: calcium, magnesium, potassium, phosphorus, and sodium; Health Canada, 1978; Health Canada, 1987a; Health Canada, 1992; Federal-Provincial-Territorial Committee on Drinking Water, 2008; WHO, 2009; Sengupta, 2013; McVean, 2019; DCCEEW, 2022; EPA, 2023; Region of Waterloo, n.d.).

This research supports the idea that freshwater mussels are natural assets, which perform important ecosystem services in the form of regulating the quality of our drinking water. Humans are increasingly aware of the deleterious effects of anthropogenic activities and the vital importance of conservation and restoration of natural systems, not only for the intrinsic existence and bequest value of these things, but because the survival and resilience of natural ecosystems and processes are essential to the continuation of life as we know it (Millenium Ecosystem Assessment, 2003; Cowie *et al.*, 2022). Therefore, as humans we should attempt to employ all of the methods at our disposal to halt or prevent large-scale human-caused environmental damage, and to restore losses where possible. Increased research efforts and the incorporation of traditional ways of knowing and Two-Eyed Seeing will allow humans to tackle these complex problems. The creation of joint management practices between indigenous communities, federal and provincial government, industry and / or institutions have shown that incorporation of indigenous knowledge has contributed to improvements in natural resource management over and over again, especially when viewed through the lens of ecosystem services (Gadgil *et al.*, 1993; Kumar, 2001; Shiferaw *et al.*, 2011; Das *et al.*, 2021). Working toward the goal of creating lasting partnerships between Mi'kmaw organizations and Cape Breton freshwater mussel researchers, workshops have been developed in partnership between researchers at Cape Breton University, Fisheries and Oceans Canada and the Unama'ki Institute of Natural Resources Guardian Program beginning in 2018 (White, 2018). Indigenous perspectives on the Eastern Pearl Mussel (*Margaritifera margaritifera* (Linnaeus, 1758)) as an indicator of the culturally significant Plamu (Atlantic Salmon, *Salmo salar* (Linnaeus, 1758)) were the basis for these workshops, and used as an educational outreach tool for researchers at Cape Breton University (K. White, personal communication, 2018).

The increasing interest in conservation in general, and for freshwater mussels in this case, is a step in the right direction. The acknowledgement of the value of diverse assemblages of lake organisms to water quality, human health, and even resilience to the effects of climate change has the potential to contribute to the conservation of freshwater mussels (Moss *et al.*, 2009; Turner *et al.*, 2009).

Understanding the effects of freshwater mussel presence on lake water and the ecosystem services they provide in terms of drinking water will be instrumental in informing policy and increasing public support for conservation efforts. This research may be used in future for public education, which may in turn increase stakeholder interest and generate a sense of investment and local value.

The concept of ecosystem services has been incorporated into nature conservation policy for nearly 20 years (Watz et al, 2022). A meta-analysis by Robinne *et al.* (2019) found 136 examples of policy documents from 46 countries which included specific references to ecosystem services published between 2004 and 2018. López-Hoffman *et al.* (2010) suggested ecosystem services as the best frame for developing policies around trans-boundary conservation between neighbouring countries, using examples such as shared groundwater reservoirs, fruit-bat pollination of crops, and protection of endangered butterflies as examples of shared interests which can lead to cooperation between nations. The International Union for the Conservation of Nature (IUCN) has developed a policy document for best practices for identification, valuation and conservation of ecosystem services provided by protected and important areas (Neugarten *et al.*, 2018). This document offers institutions and organizations a guide to assess the best tools for effective management, and ways to assess whether targets are being achieved (Neugarten *et al.*, 2018). By viewing ecosystems through the lens of the sustainability and proper functioning of essential ecological services, several aspects of conservation are automatically covered, since ecosystems are not just a collection of individual species or abiotic factors, but a complex web of interactions (Daily, 1997; Costanza and Mageau, 1999).

The ecological services of freshwater systems have been an area of particular interest, due to the human dependence on water for every area of wellbeing (Brauman, 2015; Boulton *et al.*, 2016). Ecosystem service research has been used to recommend changes in management of water resources by looking at linkages between high-intensity water use and downstream impairments in ecological services in the Mississippi River Valley in Ontario, creating a more effective and comprehensive management strategy (Retellack, 2021). Another example is the incorporation of cultural ecological services into recommendations about conservation and development decisions which may affect Atlantic Salmon (*Salmo salar*; Watz *et al.*, 2022). There have been efforts to create policy for the conservation of freshwater mussels in Europe which use the lens of ecological services as part of the justification for conservation and as a framework for decision-making (Sousa *et al.*, 2023), and there have been efforts to mobilize communication platforms like social media to increase awareness of ecological services of freshwater mussels with the aim of influencing policy (Ferreira-Rodriguez, 2022).

Water supply lakes in Cape Breton are designated as Protected Water Areas under the Nova Scotia Environment Act, which prohibits activities in the watershed such as boating, fishing, bathing, burning, use of herbicides and pesticides, waste disposal, harvesting of trees, camping, picnicking, and hunting within watersheds (Environment Act, SNS 1994; CBRM Water Utility, 2013). Current watershed management policy focuses on protecting water quality and includes an acknowledgment of the value of riparian vegetation and wetlands (Nova Scotia Environment, 2009). Future management plans should include considerations of the conservation of freshwater mussels and the species upon which they depend, and ecological services provided by freshwater mussels may be one of the justifications used for supporting policy changes in this direction.

There is an example in Cape Breton, Nova Scotia, which highlights the mutually beneficial relationship between freshwater mussels and humans. Yellow Lampmussel (*Lampsilis cariosa*) was listed as Threatened under the Nova Scotia Endangered Species Act in 2006 and listed as Special Concern under Schedule 1 of the federal Species at Risk Act (S.C. 2002, c. 29) in 2005. In 2012, Pottle Lake, a municipal water supply lake in the Cape Breton Regional Municipality (CBRM), Nova Scotia, was confirmed to house a large population of *L. cariosa*. This was a significant discovery, because Pottle Lake is one of only three known populations of the Yellow Lampmussel in Nova Scotia; the other two being Blackett's Lake and Forrester's Lake (both located within the CBRM; White, 2015; White, 2016). This discovery was also significant because Pottle Lake (a potable water source) enjoys legal protections which potentially safeguard the population of freshwater mussels within the lake.

This mutually beneficial relationship between mussels and humans in the CBRM Water Utility may be even more remarkable than at first glance. *L. cariosa* has only been observed to use White Perch (*Morone americana*) as fish hosts for their larvae (glochidia)(Haag, 2012; Fisheries and Oceans Canada, 2010; Nova Scotia Department of Natural Resources and Renewables, 2022). The fish host relationship with freshwater mussels is an important phoretic relationship without which freshwater mussels cannot reproduce (Haag, 2012). Since the time that *L. cariosa* was discovered in Pottle Lake, the threat to the population in Blackett's Lake has been elevated by the anthropogenic introduction of a major predator to White Perch, Chain Pickerel (*Esox niger* (Lesueur, 1818); White, 2023, *in prep*.). Recent research has shown much lower levels of juvenile recruitment in Blackett's Lake than in the other local populations of Yellow Lampmussel (White, 2023, *in prep.*), which may indicate that this population is unlikely to persist in this lake. Therefore, the refuge provided by Pottle Lake may have a significant role to play in the conservation of Yellow Lampmussel in Canada.

Translocation of threatened freshwater mussels has been used in many areas for conservation purposes (Cosgrove and Hastie, 2001; Haag and Williams, 2013; Nakamura *et al.*, 2022). Since freshwater mussels are found in discrete water bodies with limited connectivity such as lakes, it is essential to research the demographics of the source populations as well as the characteristics of the relocation site (such as fish host presence and water parameters) to ensure success and reduce the risk of inadvertent extirpation (Cosgrove and Hastie, 2001; Haag and Williams, 2013). Translocation may also provide conservation benefits such as increasing proliferation and diversification of mussels, which will in turn increase resilience to climate events. Translocation may also be a viable method of restoring lakes that may once have housed freshwater mussels but were sterilized via human activity in the past. Historically, copper sulphate has been used as an algicidal treatment for surface water supply lakes (van Hullebusch *et al.*, 2003; Albay *et al.*, 2003). This type of chemical treatment results in lake-wide decline of macro-invertebrates, and increased levels of copper persisting in the sediment may continue to be hostile to the development of juvenile mussels (Jacobson *et al.*, 1993; Moreno and Callisto, 2005; Satyaparameshwar *et al.*, 2006; Ray *et al.*, 2020; Dunnington and Spooner, 2019).

There are risks which must be assessed when evaluating proposed translocations of freshwater mussels, especially those that are in decline. A major consideration is the potential for transporting parasites, invasive species and diseases into new habitats with the mussels, especially when augmenting existing populations or introducing different mussel species to water bodies which already contain mussels (Brian *et al.*, 2021). However, in cases of “emergency” translocations (i.e.: when impending development threatens a known population), there have been frameworks and policies developed to guide decision making for the most beneficial methods and locations for translocation, and there have been many examples of the success of this approach (Nakamura *et al.*, 2022; Lamothe *et al.*, 2023). In the case of *L. cariosa*, I believe there is an ethical obligation to begin investigating potential translocation for the Blacketts Lake population, in order to conserve a significant proportion of known Yellow Lampmussel, which have been relegated to extirpation by anthropogenic means. Despite the longevity of individual mussels (which means adults are able to persist for decades), this situation may be considered an emergency, since the presence of *E. niger* will prevent these mussels from reproducing by causing declines in *L. cariosa*’s obligate fish host (White, 2023).

Therefore, a potential direction for future work based on my research may be to investigate the feasibility of translocating freshwater mussels into other municipally owned (and protected) water supplies with existing land-use regulations and policies. This would not only provide refugia for threatened species such as the Yellow Lampmussel, but also potentially contribute to water quality, resulting in cost savings within the water utility via reductions in chemical and mechanical treatment costs.

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