BIOFOULING IMPACTS OF AN INVASIVE MUSSEL ON NATIVE MUSSELS IN A LARGE LAKE

Brielle Comartin

Department of Biology, McGill University

Montréal, QC, Canada

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ABSTRACT

Since its introduction to North America, the Eurasian zebra mussel (*Dreissena polymorpha*) has demonstrated an ability to profoundly alter freshwater ecosystems. Through the effects of biofouling, this invasive mussel is capable of decimating populations of native mussels (Unionidae), key ecosystem engineers in lakes and rivers. However, physicochemical variables, especially calcium concentration, can limit zebra mussel invasion success and impacts on native biodiversity. In this thesis, I first summarize cases where physicochemical variables were found to limit zebra mussel biofouling of unionids, and then I report a case study that apparently challenges current risk assessments. I quantified intense biofouling and declines in local unionid population abundances in a large lake at a calcium concentration (~20 mg/L) consistently lower than levels in lakes and rivers in which unionid populations were drastically reduced following invasion. Thus, I present evidence that zebra mussels are capable of exerting significant impacts on biodiversity under environmental conditions deemed by risk assessments to be suboptimal. Overall, my research demonstrates the need for an improved understanding of the context-dependent impacts of zebra mussels.

RÉSUMÉ

Depuis son introduction en Amérique du Nord, la moule zébrée (*Dreissena polymorpha*) eurasienne a démontré la capacité à modifier pronfondément les écosystèmes d'eau douce. Par les effets de l'encrassement biologique, cette moule invasive est capable de décimer les populations de moules indigènes (Unionidae), des importants ingénieurs des écosystèmes des lacs et des rivières. Cependant, des variables physiochimiques, en particulier la concentration de calcium, peuvent limiter le succès de l'invasion de la moule zébrée et les impacts sur la biodiversité indigene. Dans cette these, je résume d'abord les cas où des variables physiochimiques ont limité l'encrassement des unionidés par la moule zébrée, puis je présente une étude de cas qui semble remettre en question les évaluations de risques actuelles. J'ai quantifié l'encrassement intense et le déclin de l'abondance des populations locales des unionidés dans un grand lac avec une concentration de calcium moyenne (~20 mg/L) inférieure aux niveaux des lacs et des rivières dans lesquels les populations d'unionidés ont été considérablement réduites après l'invasion. Ainsi, je présente des preuves que les moules zébrées sont capables d'exercer des impacts significatifs sur la biodiversité dans des conditions environnementales jugées sous-optimales par les évaluations de risques. Mes recherches démontrent la nécessité d'une meilleure comprehension des impacts dépendants du context des moules zébrées.

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CONTRIBUTION OF AUTHORS

This thesis was completed under the co-supervision of Prof. Anthony Ricciardi and Dr. Annick Drouin, who will be co-authors on the manuscript. Prof Anthony Ricciardi was involved in the conceptualization of the project and in editing drafts of the manuscript. Dr. Annick Drouin was involved in the conceptualization of the project and in execution of the fieldwork. I conducted fieldwork, analyzed the data, and wrote the manuscript. The main body of this thesis (Chapter 2) was written with the intention of journal submission.

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CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

Invasive species in freshwater ecosystems

An invasive species is an organism that is introduced to a region outside of its native range, where it establishes a self-sustaining population that becomes abundant or spreads rapidly to multiple sites, where it may cause significant socio-economic and environmental impacts (Blackburn *et al.,* 2011; Ricciardi, 2013). Among its environmental impacts, an invasive species can alter native habitats, food webs, ecosystem function (Gallardo *et al*., 2016; David *et al*., 2017) and biodiversity—through (e.g.) antagonistic interactions with native populations that can lead to their decline or extirpation (Ricciardi *et al*., 1998).

Aquatic ecosystems are particularly susceptible to being invaded, as they are foci of human activity and the vectors of aquatic species introduction are diverse and generally difficult to regulate (Ricciardi, 2006; Ricciardi & MacIsaac, 2011; Moorhouse & Macdonald, 2015). These vectors include transoceanic shipping, intentional release for cultivation and stocking, aquarium release, escape from aquaculture, and baitfish escape or release (e.g. Holeck *et al*., 2004). Consequently, lakes and rivers are among the world's most frequently invaded ecosystems (Ricciardi, 2006).

As insular ecosystems, lakes and river basins are naïve to a broad range of taxa and functional groups, and therefore are sensitive to their impacts (Cox & Lima, 2006; Dextrase & Mandrak, 2006; Ricciardi & MacIsaac, 2011). Invader impacts arise from habitat alteration (Gonzalez *et al*., 2008), as well as direct and indirect species interactions including competition, predation, herbivory, and disease transmission (e.g. Ricciardi *et al.,* 1998; Gallardo *et al.,* 2016; Koel *et al.,* 2019; Foster *et al.,* 2021). Impacts of invaders in general are highly context-dependent—i.e., they vary over space and time, owing to the influence of local biotic and abiotic environmental variables (Ricciardi *et al.,* 2013; Catford *et al.,* 2022). Furthermore, the impacts of the same invader can vary across ecosystems (e.g. Jokela & Ricciardi, 2008; Iacarella *et al.,* 2015), as well as within a heterogeneous system (e.g. Kestrup & Ricciardi, 2009), and with time since invasion (Iacarella *et al*. 2015; Karatayev *et al*., 2023).

The zebra mussel – a model invader

Among the species that have demonstrated an ability to profoundly alter freshwater ecosystems is the zebra mussel (*Dreissena polymorpha*), a freshwater bivalve native to the fresh and brackish water margins of the Black and Caspian seas (Reid & Orlova, 2002; Son, 2007). The zebra mussel was first introduced to the Great Lakes–St. Lawrence River system in the mid-1980s (Hebert *et al*., 1989), likely from the discharge of ballast water from transoceanic shipping vessels (Carlton, 2008). Zebra mussels differ morphologically from the quagga mussel (*Dreissena rostriformis bugensis*), a closely-related invasive Ponto-Caspian species, by being smaller and narrower, with a flatter base, apparently better suited to firm attachment on hard surfaces; whereas the quagga mussel, although also epifaunal, has a more rounded base (Fisheries and Oceans Canada, 2023). Zebra mussels have spread through much of eastern Canada and the USA, and are now abundant in large lakes and river systems including the lower Great Lakes, the St. Lawrence River, Lake Champlain and Richelieu River, the Hudson River, and the Mississippi River watershed (Lucy *et al*., 2014). Their invasion success has been attributed to their early maturity, high fecundity, free-swimming larval stage, and byssal attachment that allows mussels to rapidly colonize solid surfaces (Borcherding, 1991). Dense colonies of attached mussels clog intake structures in hydroelectric and power stations as well as water treatments plants, and damage watercrafts (Fisheries and Oceans Canada, 2023). The cost of zebra mussel management in Great Lakes alone exceeds $500 million per year, supporting public awareness, prevention, control and treatment activities to limit mussel colonization of human infrastructures (Vanderploeg *et al*., 2002). Zebra mussels also hinder recreational water activities such as swimming, as their shells are very sharp and capable of cutting a swimmer's feet (personal observation).

Zebra mussel larvae ('veligers') drift with water currents for several weeks before settling on virtually any solid surface available (Mackie, 1990; Borcherding, 1991; Ackerman *et al.,* 1994), and then metamorphose into mussels that attach using adhesive byssal threads (Ackerman *et al*., 1994; Rajagopal *et al.,* 1996). Female mussels begin to reproduce within 6-7 months of settling, with a single adult female capable of producing over 1 million eggs each year (Borcherding, 1991; Ackerman *et al*., 1994). The lifespan is no more than 4-5 years (Borcherding, 1991; Ackerman *et al*., 1994).

Zebra mussels are efficient filter-feeders, consuming small particles from the water column, and one single mussel can process up to a liter of water per day (Chase & Bailey, 1999). They filter phytoplankton at higher rates than native freshwater mussels, thereby reducing the amount of food particles available in the pelagic food web (MacIsaac *et al.,* 1991; Macksasitorn *et al*., 2015). The intense filtration activity of an abundant mussel population (which often exceeds that of native mussels in terms of biomass and density by orders of magnitude (Ricciardi *et al*., 1996) increases water transparency, stimulating enhanced growth of submerged aquatic plants and benthic algae (Vanderploeg *et al.,* 2001; Zhu *et al.,* 2006). Zebra mussel filtration and nutrient excretion also promotes toxic algal blooms (Bierman *et al.,* 2005; Knoll *et al*., 2008) and alters pathways for cycling contaminants and pathogens through communities (Gossiaux *et al.,* 1998; Stumpf *et al.,* 2010; Hebert *et al.,* 2014). Deposition of zebra mussel waste alters sediment quality, affecting fish spawning and egg survival due to promotion of localized anoxia (Fisheries and Oceans Canada, 2023). Zebra mussels have differential effects on the abundance and diversity of benthic macroinvertebrates, benefitting many small-bodied taxa and having antagonistic effects on some larger-bodied taxa (Ward & Ricciardi, 2013). Among their most conspicuous and severe negative impacts is on native mussels (Unionidae; hereafter 'unionids') (Ricciardi *et al.* 1998; Ward & Ricciardi, 2013).

Native unionid mussels at risk

Unionid life cycles are complex. Most species are dioecious, although some are known to be hermaphroditic (Haag, 2012). Female unionid brood fertilized eggs that develop into larvae ('glochidia') which, upon release from the female, must complete development by attaching to the gills, fins, or body of a passing fish (or, in rare cases, a salamander); this parasitic/commensal relationship between unionid species and host fishes can range from generalist to highly specific, and glochidia are generally harmless to their host fish (Wächtler *et al.,* 2001). It can take 2–9 years for juveniles to become sexually mature within a life span of 15–40 years or more under ideal conditions (Haag, 2012).

Unionids typically burrow into the substrate, exposing the posterior end of their shells to the water column to conduct major functions including feeding, respiration and excretion, by drawing water in through their incurrent siphon, moving water over their gills and then passing it out through their excurrent siphon (Haag, 2012). Among the retained food particles are phytoplankton, small zooplankton, bacteria, and organic detritus (Vaughn *et al.,* 2008). Their activities cause unionids to serve influential roles in lake and river ecosystems. Their burrowing mixes sediments and alters their physicochemical properties (McCall *et al.,* 1979; McCall *et al.,* 1986). Through filter-feeding the water column, unionids transfer organic matter from the water column and excrete nutrients that nourish the local benthos (Strayer 2014; Vaughn *et al*. 2004; Pusch *et al*. 2001). They also remove particles (including those bearing contaminants) and organisms that can harm water quality (Kim *et al.,* 2011; Atkinson *et al.,* 2013), while simultaneously absorbing heavy metals and other contaminants (Smith *et al.,* 1975). Unionid shells provide physical habitat for macroinvertebrates, small fish, and salamander larvae (Vaughn & Spooner, 2006). When unionids are present in large numbers, mussel beds support diverse species and attract fish to feed, including their host fishes (Ilarri *et al.,* 2018; Modesto *et al.,* 2018). They also serve as important food sources for various fishes, waterfowl, river otters, raccoons, and muskrats (Zahner-Meike & Hanson, 2001). Unionids are also culturally significant to Native Americans, who used the shells as decorations and tools, and to European colonists, who used the nacre of mussel shells for making buttons and now commonly to obtain pearls (Haag, 2012).

Historically, North America has the richest freshwater mussel fauna in the world, with close to 300 described species and subspecies (Williams *et al.,* 1993). However, North American unionids are among the most imperiled aquatic animal groups on the planet, owing to habitat degradation in the form of chemical pollution, siltation, stream channelization and impoundment (Bogan, 1993; Williams *et al.,* 1993). At least 70% of North American unionids are either extinct, endangered, threatened or listed as special concern (Williams *et al.,* 1993; Haag & Williams, 2014). The zebra mussel invasion has added a major threat to North American unionid populations.

Impacts of zebra mussels on native mussels

Having evolved in the absence of dominant fouling organisms, North American unionid mussels have no adaptive mechanisms to resist the effects of zebra mussel attachment and colonization (i.e. biofouling) of their exposed shells (Ricciardi *et al.,* 1998). Heavy zebra mussel biofouling may impair unionids by smothering their siphons used for metabolic activities such as filter feeding, respiration, and excretion, thereby causing suffocation and starvation (Lewandowski, 1976; Mackie, 1990; Schloesser & Kovalak, 1991). For example, fouled unionids have been found to experience a reduction in oxygen uptake and increased grazing rates from increased respiration and nutritional stress, respectively (Baker & Hornbach, 1997). A large mass of attached zebra mussels weighing down the unionid shell can impose further energetic costs, causing increased energy expenditure to maintain the proper upright orientation to perform metabolic activities (Haag *et al*., 1993; Schloesser & Nalepa, 1994; Ricciardi *et al*., 1996). Zebra mussel fouling reduces the amount of stored glycogen in unionids (Patterson *et al*., 1997; Hallac & Marsden, 2000; Beason & Schwalb, 2022), which could result in insufficient energy stores to survive the winter in high-latitude regions (Ricciardi *et al*., 1996). Furthermore, zebra mussel fouling limits unionid locomotion by preventing extension of the foot and proper opening/closing of the shell valves, potentially disrupting reproduction, predator avoidance and the ability to escape unfavorable environments (Appledorn & Bach, 2007).

The susceptibility and tolerance to zebra mussel biofouling varies among unionid species. One reason for this may be differences in breeding strategies, where unionids that spend more time and energy on reproduction experience greater mortality than short-term brooders (Schloesser *et al*., 1997; Graf & Foighil, 2000), likely reflecting the higher energetic costs of persistently sustaining glochidia over longer periods (Hallac & Marsden, 2000). In sexually dimorphic unionid species, females may be more sensitive than males to zebra mussel fouling (Haag *et al*., 1993; Hallac & Marsden, 2000). Species-specific differences may be an effect of shell characteristics such as surface properties, where the microtopology and chemical composition of some unionid shells may be less favorable for zebra mussel attachment (Dzierżyńska-Białończyk *et al.,* 2018). Additionally, unionid species with thicker and heavier shells may be more tolerant to fouling than those with lighter shells, perhaps because of greater musculature that can be used to overcome interference across the gape of the shell (Haag *et al*., 1993). Burrowing behavior of unionid species could be another reason for these differences, as some unionids are capable of burrowing to greater depths than others, leaving less shell area exposed and vulnerable to zebra mussel settlement (Nichols & Wilcox, 1997; Sousa *et al.,* 2011; Bódis *et al.,* 2014).

Zebra mussels could preferentially attach onto unionids over other hard substrata. Zebra mussels attached to unionids have been found to have higher growth rates than those attached to rocks, possibly by intercepting food from the siphonal current of unionids (Hörmann & Maier, 2006), as well as enhanced body condition (body mass and glycogen content) than those settled on inanimate substrates (Pilotto *et al.,* 2016). The fouling impact of zebra mussels on unionids is largely dependent on various local environmental factors that can significantly influence its severity.

Physicochemical mediation of zebra mussel impacts

Local physicochemical variables such as temperature, substrate size, lake depth, pH and calcium concentration can limit zebra mussel distribution and population density—and therefore its impacts on native biodiversity (Table 1.1). The acute lethal threshold of zebra mussel thermal tolerance has been reported as 30°C (Iwanyzki & McCauley, 1993; Garton *et al*., 2014), although mass zebra mussel mortalities have been reported at temperatures near ~25°C (White *et al*., 2015). Temperatures above the thermal tolerance for zebra mussels may provide a refuge from biofouling for some unionid taxa, as suggested by one study that recorded higher unionid diversity and abundance with lower rates and intensities of fouling within a thermal plume of a power plant (Bryan *et al*., 2013; 2014). Optimal pH values for zebra mussels have been typically reported around 8.0 (Neary & Leach, 1992; Claudi & Mackie, 1994;); however, pH is a better predictor of zebra mussel occurrence when combined with other water chemistry variables such as calcium concentration (Hincks & Mackie, 1997; see below). Zebra mussel abundance is maximal on large stable substrates (such as boulders and bedrock) in shallow, alkaline waters (Mellina & Rasmussen, 1994; Jones & Ricciardi, 2005).

Nearshore embayments and coastal wetlands can offer important refuge for unionids (Nichols & Amberg, 1999; Zanatta *et al*., 2002; Larson *et al*., 2022). Their characteristics include soft sediments that allow unionids to burrow, removing attached zebra mussels (Nichols & Wilcox, 1997; Schloesser *et al*., 1997), and water-level fluctuations from seiche events or seasonal changes that expose zebra mussels to shallower depths (Bowers & de Szalay, 2004; 2005). Some other factors that reduce zebra mussel biofouling include predation from molluscivorous fish on zebra mussels attached to unionids (Bowers *et al*., 2005; Bowers & de Szalay, 2007), warm thermal plumes (Bryan *et al*., 2013; 2014), and quagga mussel-dominated waterbodies (Burlakova *et al*., 2014) (Table 1.1).

Calcium concentration is arguably the most important water chemistry variable for predicting zebra mussel distribution, abundance and impact. Calcium concentrations at or above 25 mg/L are considered optimal for zebra mussels (Therriault *et al*., 2013), allowing them to exert strong negative ecological impacts on unionids (Nalepa, 1994; Schloesser & Nalepa, 1994; Ricciardi *et al*., 1996; Hallac & Marsden, 2000; Martel *et al*., 2001). Zebra mussel biomass increases with calcium up to a concentration of 25 mg/L, after which other variables (such as substrate, depth and pH) exert a greater influence (Jones & Ricciardi, 2005). Similarly, zebra mussel fouling intensity on unionids is strongly positively correlated with calcium concentration, increasing with calcium concentration up to an asymptotic threshold of at least 24 mg/L (Jokela & Ricciardi, 2008). Zebra mussel survival is negligible at calcium concentrations below 10-12 mg/L (Jones & Ricciardi, 2005; Hincks & Mackie, 1997), and is possible at concentrations between 12-15 mg/L with limited reproduction (Therriault *et al*., 2013). Calcium concentration limits shell production and growth, and osmoregulation in zebra mussels (Vinogradov *et al.,* 1993), whereas unionids are much more tolerant to low-calcium conditions (as low as 2-3 mg/L; Strayer et *al*., 1981; Rooke & Mackie, 1984; McMahon & Bogan, 2001). Therefore, low calcium conditions may provide refugia for unionid mussels from the impacts of zebra mussels (Hasewaga & Maekawa, 2006; Jokela & Ricciardi, 2008).

Unionid mortality associated with zebra mussel biofouling has been reported in some lakes (Mellina & Rasmussen, 1994; Jokela & Ricciardi, 2005; Kirkendall *et al*., 2022) and rivers (Cusson & De Lafontaine, 1997; De Lafontaine & Cusson, 1997; Eliopoulous & Stangel, 2000) where calcium concentrations are suboptimal for zebra mussel establishment (<20 mg/L). However, zebra mussel populations in these waterbodies are likely not reproducing *in situ*, but rather originate from an upstream source of zebra mussel veligers with higher calcium concentrations where reproduction is possible. Thus, upstream donor populations, where physicochemical conditions are suitable, act as a “source populations” for the receiving “sink populations”, in areas where zebra mussel establishment would not be attainable otherwise due to limiting environmental factors (Horvath *et al*., 1996).

At suboptimal calcium concentrations between 15–20 mg/L, it is debatable whether zebra mussels can reproduce sufficiently to sustain populations (Hincks & Mackie, 1997; Cohen & Weinstein, 2001; Jones & Ricciardi, 2005; Whittier *et al*., 2008). Cases of high abundances of zebra mussels in suboptimal calcium waters have been reported, although these were likely due to the recruitment of veligers carried downstream from source populations in higher calcium waters upstream (reviewed in Cohen & Weinstein, 2001). Lethal fouling intensities on unionids may occur at sites with suboptimal calcium concentrations if an upstream source of larvae is available and if unionids are the principal source of colonizable substrate in the area (Jokela & Ricciardi, 2008). Field data are lacking to suggest that zebra mussels can sustain a population capable of exerting strong impacts on unionids in inland waterbodies with suboptimal calcium concentrations where they are reproducing *in situ*.

Research objectives

The goal of this research was to quantify the magnitude and variation of zebra mussel biofouling on native unionids over space and time in Lake Memphrémagog, Québec, a lake with suboptimal calcium (~20 mg/L) representing a closed system in terms of zebra mussel population dynamics. Zebra mussels became established in Lake Memphrémagog by 2017. Contrary to published risk assessments predicting low densities and constrained impacts at suboptimal calcium concentration, zebra mussel densities increased by 5-fold at some sites in the lake by 2020, attaining abundance levels as high as those recorded in calcium-rich waterbodies (i.e. $[Ca^{2+}] \geq 25$ mg/L) in which they have exerted strong biofouling impacts. My objectives were to determine the status of local unionid populations after being recently subjected to zebra mussel biofouling and to determine if fouling was correlated with site-level calcium concentration, and compare these data to those recorded in calcium-rich waterbodies at a similar stage of invasion.

Tables

Table 1.1. Physicochemical variables found to limit zebra mussel biofouling of unionids.

Limiting variables	Context (example)	Impact on zebra mussels & fouling intensity	Impact on unionids	References
Temperature	Thermal plume	Lower densities and fouling intensities within the thermal plume	Higher density and diversity within the thermal plume	Bryan *et al*., 2013; 2014
Calcium concentration	Suboptimal calcium for zebra mussels	Low densities and fouling intensities than in calcium-rich water	Higher density and diversity in areas where zebra mussels are calcium-stressed	Mellina & Rasmussen, 1994; Jones & Ricciardi, 2005; Jokela & Ricciardi, 2008
Hydrological (water depth; wave action)	Coastal wetlands	High numbers of veligers but lower fouling intensities than at offshore sites	Species richness comparable to offshore areas in lower Great Lakes prior to zebra mussel establishment	Nichols & Amberg, 1999; Zanatta *et al*., 2002
Unionid burrowing behavior in silt-clay sediments	Silt-clay sediments within a wetland	Low densities and intensities of fouling compared to coarse substrates	High diversity in wetlands; no unionids found in coarse, sand-gravel substrates	Nichols & Wilcox, 1997; Schloesser *et al.,* 1997
Primary productivity	Nearshore bays	Low densities and intensities of fouling compared to open-waters and river mouths	Moderate positive correlation between unionid growth & biofouling; likely due to high primary productivity affecting all mussels	Larson *et al.,* 2022
Aerial exposure	Dewatering event	Low densities of veligers and adults in shallow water	Densities comparable to other coastal wetlands	Bowers & de Szalay, 2004, 2005
Predation	Cages limiting fish predation	Higher densities and intensities of fouling inside cages than outside	Same densities inside and outside of cages	Bowers *et al*., 2005; Bowers & de Szalay, 2007
Competitive exclusion	Waterbodies dominated by quagga mussels	Lower fouling intensities compared to zebra mussel-dominated waters	Hypothesized to be reduced, but no differences documented to date	Burlakova *et al.,* 2014

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CHAPTER 2: IMPACTS OF ZEBRA MUSSEL (DREISSENA POLYMORPHA) INVASION ON NATIVE MUSSELS (UNIONIDAE) IN AN INLAND LAKE WITH SUBOPTIMAL WATER CHEMISTRY

Abstract

Invasions by the Eurasian zebra mussel (*Dreissena polymorpha*) is a driver of declines and even extirpations of native unionid mussel populations in some North American lakes and rivers. Water chemistry, particularly calcium concentration, is used to predict waterbodies that are at risk of invasion and impacts by zebra mussels. Calcium concentrations ≥ 25 mg/L are considered optimal for supporting dense zebra mussel populations that can exert severe biofouling impacts native species. Current risk assessments generally consider concentrations of 15-20 mg/L as suboptimal, and the literature is equivocal about whether zebra mussels can sustain populations under such conditions in lakes without an upstream source of larvae. Here, we surveyed zebra mussel fouling intensities and unionid mortality across sites and over time in Lake Memphrémagog (Quebec), a waterbody with an average calcium concentration of ~20 mg/L. Despite anticipated calcium limitations, the zebra mussel reproduces *in situ* within this closed system and has achieved local population densities comparable to those associated with unionid mortality in calcium-rich waterbodies. Our results indicate that zebra mussel has caused significant fouling and mortality in unionid populations approximately 7 years post-invasion. We hypothesize that suboptimal water chemistry has delayed the onset of lethal fouling compared to calcium-rich systems. This case underscores the need for risk assessment to provide more nuanced consideration of how the impacts of zebra mussels on native biodiversity vary with habitat conditions.

Introduction

The zebra mussel (*Dreissena polymorpha*) is an invasive Eurasian bivalve that can rapidly restructure freshwater benthic communities. Among the taxonomic groups most negatively affected by invasion in North American are native mussels (Unionidae; hereafter "unionids") (Nalepa *et al.,* 1996; Schloesser *et al.,* 1996; Strayer & Malcom, 2007a). Given that North American unionid mussels have evolved in the absence of dominant fouling organisms, they have no adaptive mechanisms to resist the effects of zebra mussel biofouling—in which zebra mussels colonize and overgrow their exposed shells (Lewandowski, 1976; Strayer & Smith, 1996; Ricciardi *et al*., 1998). Intense zebra mussel fouling can impair unionids by smothering their siphons, thereby interfering with vital activities (feeding, respiration, reproduction, and excretion) and ultimately causing suffocation or starvation (Mackie, 1990; Schloesser & Kovalak, 1991; Burlakova *et al.,* 2000). Furthermore, the burden of a large mass of attached zebra mussels imposes an energetic cost for a host unionid to maintain its proper orientation in the substrate necessary for filtration and metabolic activities (Haag *et al*., 1993; Schloesser & Nalepa, 1994; Hallac & Marsden 2000). Zebra mussel biofouling of unionids is thought to be a major driver of recent declines of unionid populations in North American lakes and rivers, which have been recorded within 3 to 6 years post-invasion (e.g., Nalepa, 1994; Ricciardi *et al*., 1996; Ricciardi *et al*., 1998; Martel *et al*., 2001; Schloesser *et al.,* 2006). Severe unionid mortality (> 90%) is predicted to occur when mean biofouling levels reach 100 zebra mussels/unionid (Ricciardi *et al.,* 1995), or when the mass of the attached zebra mussels equal or exceed that of their unionid host (i.e. mass ratio ≥ 1) (Martel *et al*., 2001; Ricciardi, 2003). Unionids are species-specific in terms are their susceptibility and tolerance to zebra mussel biofouling (Haag *et al*., 1993; Ricciardi *et al*., 1996; Hallac & Marsden, 2000). Furthermore, spatial variation in impacts in heterogenous habitat conditions has been observed, creating refugia for native unionid mussels (Zanatta *et al*., 2002; Jokela & Ricciardi, 2008; Hollandsworth *et al*., 2011).

Calcium concentration is a key water chemistry variable used to predict waterbodies that are at risk of zebra mussel invasion and its impacts on native species (Jokela & Ricciardi, 2008). Zebra mussel densities increase with calcium up to a concentration of 25 mg/L, after which other variables (substrate, depth, and pH) can exert a greater influence (Mellina & Rasmussen, 1994; Jones & Ricciardi, 2005). At calcium concentrations below 10–12 mg/L, there is virtually no possibility of zebra mussel survival or reproduction (Hinks & Mackie, 1997; Jones & Ricciardi, 2005). At calcium concentrations between ~12–15 mg/L, zebra mussel survival is possible, but reproduction is very limited (Therriault *et al*., 2013). Between 15 and 20 mg/L, conditions are suboptimal, and it is debatable whether zebra mussels can sustain populations without an upstream source of larvae (reviewed in Cohen & Weinstein, 2001). Calcium concentrations of at least 25 mg/L are considered optimal for the reproduction and survival of dense zebra mussel populations (Therriault, *et al.,* 2013), and it is such alkaline waterbodies that zebra mussels have exerted strong negative impacts on unionid populations (Nalepa, 1994; Schloesser & Nalepa, 1994; Ricciardi *et al*., 1996; Hallac & Marsden, 2000; Martel *et al*., 2001).

The zebra mussel established in Lake Memphrémagog (Québec) circa 2017 (Picard & Doyon, 2018; Gosselin-Bouchard & Godmaire, 2020). By 2019, the mean densities of zebra mussels were 1.8 m^{-2} in Lake Memphrémagog, 3.76 m^{-2} in the downstream Lake Magog, and 0.32 m^{-2} in the Magog River which connects both lakes (D’Avignon *et al*., 2020). By this time, some zebra mussels had already begun to foul unionids (D’Avignon *et al*., 2020). However, it was predicted that zebra mussels would not attain densities sufficiently high to severely reduce native mussels, owing to a suboptimal average calcium concentration in Lake Memphrémagog ($\leq$ 20 mg/L; Picard & Doyon, 2018; D'Avignon *et al.,* 2020; Gosselin-Bouchard & Godmaire, 2020). The Québec government categorizes Lake Memphrémagog as a "medium" risk of zebra mussel establishment (Goulet & Normand, 2023). However, despite anticipated calcium limitations, mean zebra mussel densities increased exponentially to 1791 m^{-2} by 2022 in some parts of the lake (MELCCFP, 2022), comparable to densities associated with unionid mortality in calcium-rich waterbodies (Ricciardi *et al*., 1995). Lake Memphrémagog is a heterogenous lake representing a closed system in terms of zebra mussel population dynamics, with reproduction occurring *in situ* and where veligers are assumed to be distributed downstream to the Magog River and Lake Magog (D'Avignon *et al*., 2019) through the northerly currents (Larocque & Dubois, 2012). This case could thus provide valuable field evidence that the zebra mussel is capable of exerting significant impacts on native biodiversity over a broader range of environmental conditions than currently documented.

The objectives of this research are 1) to quantify the rate and magnitude of change of zebra mussel biofouling on native unionids across sites and over time in Lake Memphrémagog; 2) determine the status of the unionid population as a consequence of biofouling in the connected downstream waterbodies, the Magog River and Lake Magog; and 3) compare these data to those recorded in calcium-rich waterbodies at similar stages of invasion. Specifically, we tested the hypotheses that 1) fouling intensity is correlated with site-level calcium concentration and 2) observed changes in unionid mortality (and thus population density) are correlated to fouling intensity.

Methods

Study location and sampling

Lake Memphrémagog is a long (50 km), narrow (1 to 4 km) and deep (<107 m) freshwater glacial lake located between Newport, Vermont (USA) and Magog, Québec (Canada). Most of the lake's surface area is in Québec, whereas three-quarters of the lake's watershed is in Vermont. Three rivers drain into Lake Memphrémagog at its extreme southern end, providing the primary input of nutrients into the lake from agricultural runoff. Most of the lake drains into the Magog River in Québec, which connects to Lake Magog and flows into the Saint-François River in the city of Sherbrooke. The lake can be divided topographically into three distinct areas defined by a deep central basin and shallower north and south basins (Larocque & Dubois, 2012).

Sampling was conducted over two years (2022-2024) at a total of 19 sites in Lake Memphrémagog, Magog River and Lake Magog (Figure 2.1). Three sites in the northern basin of Lake Memphrémagog were sampled in the Fall 2022 (September 26 – October 1) and Spring 2023 (June 2–3), and Fall 2023 (October 13) and Spring 2024 (June 3–7) to assess unionid population mortality from biofouling over two winters (Supplementary Materials; Table S2.1). Only three sites were selected owing to logistical reasons. All other sites were sampled in Summer 2023 (July 24–27) and Summer 2024 (July 1–15) during peak zebra mussel settlement, except for sites in the southern basin of Lake Memphrémagog, the Magog River and Lake Magog, which were sampled only in Summer 2024 to investigate how zebra mussel fouling has spread throughout the waterbody (Table S2.1).

All sites (depth 1–6m) were accessed either from shore or by boat and were sampled manually by a SCUBA diver. At every site, all unionids, including empty shells, were removed by hand from a 1-m^2 polyvinyl chloride quadrat cast haphazardly on the bottom substrate; specimens were places in sealed 5-mm mesh bags before being brought to the surface. The upper 10 cm of sediment within the quadrat was probed by hand to locate all individuals buried immediately beneath the surface. It was decided *a priori* to use five replicated 1-m^2 quadrats at each site, as this was expected to yield a precision of ±20% when sampling unionid densities of 10-30 mussels/m^2 (Downing & Downing, 1992). When few (< 10) or no unionids were collected within the first five quadrats, an additional five quadrats were sampled. All unionid species were identified in the field following criteria described by Clarke (1981) and McMahon (1991) and verified using the Clam Counter app (version 1.74). Images were captured of every unionid after being brought to the surface and cleaned of zebra mussels, to aid with species identification. Recently dead unionids were distinguished from older shells by their intact hinge ligament and uneroded nacre, following Ricciardi *et al.* (1996). All live unionids were weighed (g) on a digital scale with and without zebra mussels attached, providing a measure of mass ratio, and their shell length (cm) was determined using a measuring board. After processing, live unionids were returned to their original location.

Zebra mussels were removed from each unionid using forceps, placed in a labelled whirl-pack bag, and stored in a cooler to be transported to the laboratory where they were counted, providing a measure of fouling intensity. To better understand the colonization and rate of reproduction of zebra mussels across the lake, shell lengths (mm) of zebra mussels collected during the 2024 sampling year were measured using digital calipers. Although there can be several breeding periods during the summer that produce different cohorts, a shell length of 1 to 15 mm is usually recognized to correspond to young-of-the-year individuals (D'Avignon *et al.*, 2020); however, depending on the body of water, certain zebra mussels mature when they reach a size between 7-9 mm (D'Avignon *et al*., 2020). Taking into account the environmental conditions and the period during which the samples took place in Lake Memphrémagog and connected waters, it was decided that a size of <10 mm constitutes a juvenile mussel.

Physicochemical variables

A multiparameter YSI Pro Solo meter was used to assess water temperature (°C), pH, specific conductivity (k Ω cm), and total dissolved solids (g/L), at each sampling site. Weather conditions and air temperature (°C) were also recorded. Replicate water samples were taken underwater in 1-L plastic bottles at each site, which were transported to the lab in a cooler and kept refrigerated until examined in the laboratory. Calcium and magnesium concentrations (mg/L) were determined by titration using a LaMotte Hardness Test, chlorophyll-*a* concentration (*u*g/L) was estimated using a spectrophotometer, and turbidity (NTU) was measured using a turbidimeter. At every site, the percentage of submerged vegetation was estimated (within a 1-m^2 quadrat), and the mean diameter (in mm) of the sediment was visually estimated and converted into a phi value, i.e. $\phi = -\log_2$ (diameter, mm), following the Wentworth scale (Mellina & Rasmussen, 1994; D'Avignon *et al*., 2020). An underwater camera was used to take images of each sampling site, for visualization of the surrounding environment.

Statistical analysis

Statistical analyses were conducted in RStudio (version 4.4.1). Data were tested for normality using a Shapiro-Wilk test and for homogeneity of variances using either an F-test or Levene's test.

A chi-squared test was used to test for over- and under-representation of unionid species in samples of recently dead individuals for all sampling years (2022, 2023 and 2024). A one-way ANOVA was used to test for differences in mean unionid density and mean biofouling metrics (fouling intensity and mass ratio) across sites within each summer sampling year in Lake Memphrémagog (2023 and 2024) and across waterbodies for the 2024 sampling year. If normality or equal variance assumptions were violated, a Welch's ANOVA or a Kruskal-Wallis rank sum test was used, respectively. Chi-squared tests were used to determine differences in recent unionid mortality across sites within each summer sampling year in Lake Memphrémagog and across waterbodies for the 2024 sampling year. A Games-Howell post-hoc test was conducted to compare combinations of sites that were significantly different from one another.

A Principal Component Analysis was conducted to define groups of sites in each summer sampling year in Lake Memphrémagog by recent unionid mortality, mean unionid density and mean fouling intensity, based on physicochemical variables. A simple linear regression analysis assessed the relationship between the mean fouling intensity and the mean calcium concentration across these sites; the data were averaged and compared to a published model for various zebra mussel invaded habitats (Jokela & Ricciardi, 2008). An ANCOVA was conducted to test whether the relationship between the mean mass ratio and recent unionid mortality differed between Lake Memphrémagog (where $[Ca^{2+}]$ = ~20 mg/L) and calcium-rich North American waterbodies (where $[Ca^{2+}]$ = ~30 mg/L), using data from all years and seasons from Lake Memphrémagog, and data from a published model (Ricciardi, 2003), respectively.

A paired two-sample t-test was used to compare the unionid population status (mean unionid density and recent unionid mortality) and biofouling metrics (mean fouling intensity and mean mass ratio) in the summer in Lake Memphrémagog across two consecutive years (2023-

2024). If normality or equal variance assumptions were violated, a paired two-sample Wilcoxon test was performed. A repeated-measures ANOVA test was used to compare the unionid population status and biofouling metrics across seasons in Lake Memphrémagog (Fall 2022, Spring 2023, Fall 2023, Spring 2024) to test for a correlation with overwinter mortality.

Finally, a simple linear regression analysis was used to assess the relationship between the recent unionid mortality in 2024 and mean mass ratio in 2023 for data collected in the summer in Lake Memphrémagog.

Results

Unionid population status

Overall sampling at various sites over two years revealed the presence of at least three unionid species: *Elliptio complanata* (95.4%), *Lampsilis radiata* (3.3%) and *Pyganodon* sp. (1.3%) (percentages based on a total of 2,610 live individuals). A total of 180 empty shells were collected, of which 150 were recently dead individuals and 30 were older shells. Annual totals are as follows. In 2022, we collected 253 live individuals (94.9% *E. complanata*, 3.6% *L. radiata*, 1.6% *Pyganodon* sp.) and 7 recently dead individuals (71.4% *Elliptio complanata*, 14.3% *L. radiata,* 14.3% *Pyganodon* sp.) (Figure S2.1). In 2023, we collected 1,097 live individuals (96.6% *E. complanata,* 2.4% *L. radiata*, 1.0% *Pyganodon* sp.) and 47 recently dead individuals (76.6% *E. complanata*, 14.9% *L. radiata*, 8.5% *Pyganodon* sp.) (Figure S2.2). In 2024, we collected 1,260 live individuals (94.4% *E. complanata,* 4.1% *L. radiata*, 1.5% *Pyganodon* sp.) and 96 recently dead individuals (91.7% *E. complanata*, 5.2% *L. radiata*, 3.1% *Pyganodon* sp.) (Figure S2.3). One species, *E. complanata*, was underrepresented in samples of recently dead mussels compared to the live ones in 2022 (Pearson's chi-squared: $X^2(2) = 8.1524$, $p = 0.01697$) and 2023 ($X^2(2) = 45.767$, $p < 0.0001$); *E. complanata* was present at all sites in all waterbodies, whereas *L. radiata* and *Pyganodon* sp. were present at only a few sites in Lake Memphrémagog and not found in the Magog River or Lake Magog.

Variation in unionid abundance and biofouling across sites and waterbodies

Unionid density differed across sites in Lake Memphrémagog in summer 2023 (Kruskal-Wallis: $X^2(7) = 36.351$, $p < 0.0001$; Figure S2.4A) and in summer 2024 ($X^2(10) = 43.604$, $p < 0.0001$; Figure S2.4B), whereas the proportion of recently dead unionids differed across sites in 2024 ($X^2(10) = 69.159$, $p < 0.0001$; Figure S2.5B), but not in 2023 ($X^2(7) = 10.879$, $p = 0.144$; Figure S2.5A). Biofouling also varied across sites in summer 2023 (fouling intensity: $X^2(7) = 305.36$, $p < 0.0001$; Figure S2.6A; mass ratio: $X^2(6) = 105.02$, $p < 0.0001$; Figure S2.7A) and summer 2024 (fouling intensity: $X^2(10) = 551.98$, $p < 0.0001$; Figure S2.6B; mass ratio: $X^2(9) = 112.12$, $p < 0.0001$; Figure S2.7B).

When comparing across waterbodies for 2024 summer data, unionid density did not vary significantly among Lake Memphrémagog, Magog River and Lake Magog ($X^2(2) = 3.4185$, $p = 0.181$; Figure S2.8A). However, recent mortality ($X^2(2) = 18.128$, $p < 0.0001$; Figure S2.8B) and biofouling levels (fouling intensity: Welch's one-way ANOVA: $F(2) = 67.501$, $p < 0.0001$; mass ratio: $F(2) = 160.7$, $p < 0.0001$; Figure S2.9) differed across these three waterbodies. Physicochemical variables collected from Lake Magog and the Magog River are presented in Table 2.1.

Evidence of continued fouling due to larval settlement is indicated by the presence of juvenile zebra mussels on shells of live unionids at 10 sites in Lake Memphrémagog, Magog River and Lake Magog in 2024. Sites can differentiated into two groups by the total numbers of attached zebra mussels on the live unionid assemblage (Figure 2.2).

The variation in unionid abundance and biofouling across sites in Lake Memphrémagog was not conspicuously influenced by physicochemical factors in either summer sampling year, as the sites could not be grouped along the PC1 and PC2 axes (Figure S2.10). Two sites in the southern basin (Cedarville and Île Ronde) were excluded from the 2024 analysis because of some missing physicochemical variables. All physicochemical data are presented in Table 2.1.

Biofouling as a function of calcium concentration

Over the calcium range measured across summer sites in Lake Memphrémagog (17.4–26.6 mg/L), there was no relationship with mean fouling intensity in 2023 ($R^2 = 0.007$, $p = 0.848$) or 2024 ($R^2 = 0.037$, $p = 0.572$). When data are averaged for 2023 and 2024, the relationship between mean fouling intensity and calcium concentration fits an empirical model of data from other invaded systems (Figure 2.3). However, the relationship between the mean mass ratio and recently dead unionids across all data for Lake Memphrémagog differs from that of data from calcium-rich (≥30 mg/L) habitats in North America (ANCOVA: $F(1,54) = 65.96$, $p < 0.0001$; Figure 2.4).

Unionid abundance across years and seasons

Data collected over two summers in Lake Memphrémagog showed a significant rapid increase in the proportion of recent dead unionids (paired two-sample t-test: $t(7) = -2.744$, $p = 0.014$; Table 2.2; Figure 2.5A) suggesting a change in mortality. However, there was no significant declines in unionid density averaged over all sites from 2023 to 2024 (paired two-sample Wilcoxon: $V(7) = 18$, $p = 0.527$; Table 2.2; Figure 2.5B), although some individual sites experienced significant declines between the years (Table 2.2). Density data collected in Summer

2024 from the southern basin of Lake Memphrémagog, the Magog River and Lake Magog were not included in this temporal comparison but are shown in Table 2.2.

Data collected over two falls and two springs in Lake Memphrémagog did not reveal any overall trend in recent unionid mortality (repeated measures ANOVA: F(3,6)= 1.143, p = 0.405; Table 2.3) nor in mean unionid density (F(3,6) = 0.271, *p* = 0.844; Table 2.3).

Biofouling and unionid mortality across years and seasons

There were no significant trends in biofouling for data collected across seasons: mean fouling intensity (F(3,6) = 1.317, *p* = 0.353; Table 2.5), mean mass ratio: (F(3,6) = 1.963, *p* = 0.221; Table 2.5). From Summer 2023 to Summer 2024, there was no significant change in mean fouling intensity as measured by the mean number of zebra mussels per unionid (V(7) = 12, *p* = 0.231; Table 2.4; Figure 2.6A), although some individual sites experienced significant increases in fouling intensity between years (Table 2.4). Similarly, the mean mass ratio (g zebra mussels/g unionid) did increase during this time (t(7) = -2.671, *p* = 0.016; Table 2.4; Figure 2.6B). Data collected in Summer 2024 from the southern basin of Lake Memphrémagog, as well as the Magog River and Lake Magog were not included in this analysis (but are shown in Table 2.4). Nevertheless, there was a significant relationship between recent unionid mortality in 2024 and mean mass ratio in 2023 (F(1,6) = 11.3 , R^2 = 0.65, *p* = 0.015; Figure 2.7).

Discussion

Unionid population status

Most unionids collected in Lake Memphrémagog were *Elliptio complanata*, the most common and widespread species in northeastern North America (William *et al.,* 2017). This species was also the only one collected in Lake Magog and the Magog River. Two other common species in this biogeographical range, *Lampsilis radiata* and *Pyganodon* sp., were collected from the lake system, but in much lower abundances than *E. complanata.* Sporadic unionid inventories in Lake Memphrémagog between 1996-2002 indicated the presence of another species, *Anodontoides ferussacianus*, in 2002 (Normand & Goulet, 2022); however, this species was not observed in a more recent survey (D'Avignon *et al*., 2020), nor in this study, and so the 2002 report might have been based on a misidentified *Pyganodon* specimen. The lack of regular unionid monitoring data over the years could have resulted in an absence of records for rare species or recently extirpated species. However, the same three taxa identified in Lake Memphrémagog were also reported in the neighboring Lake Massawippi (Bleu Massawippi, 2023), further suggesting that they are the most common species in the area.

Relative population status can perhaps be explained, at least in part, by life history strategy. Unionid taxa that become highly abundant are typically those with long lifespans and late maturity, falling under the equilibrium life history strategy that is further characterized by low fecundity (Haag, 2012); these species include *Elliptio complanata* (Moore *et al.,* 2021). The opportunistic strategy comprises unionid taxa with short lifespans, early maturity and high fecundity, such as *Pyganodon* (Haag, 2012). Between these two contrasting strategies is the periodic strategy, in which species have moderate-to-high growth rates and low-to-intermediate lifespans, age at maturity, and fecundity, as characterizes most *Lampilis* species (Haag, 2012). However, life history strategies alone do not fully explain why some species dominate assemblages; competition for resources can also play a structuring role. For example, owing to metabolic differences associated with growth rate, long-lived and slow-growing equilibrium species such as *E. complanata* (Moore *et al*., 2021) have lower resource requirements and therefore may dominate over fast-growing opportunists (*Pyganodon* spp.) and periodics, when food is limiting (Haag, 2012; Moore *et al.*, 2021). Further, opportunistics typically have thinner shells, which may make them poor competitors for space in dense mussel beds (Haag, 2012). With respect to fouling, species with lighter, thinner shells (such as *Pyganodon*) may be more detrimentally affected by the burdensome mass of attached zebra mussels than species with relatively thicker shells (such as *E. complanata*), which are able to more effectively regulate their center of gravity when encumbered by the additional weight (Haag *et al.*, 1993; Gillis & Mackie, 1994; Martel *et al.*, 2001).

Although *E. complanata* was the most abundant species in all samples of live and dead individuals*,* it was underrepresented in samples of recently dead unionids in 2022 and 2023. In Lake Memphrémagog, *E. complanata* shells could conceivably have degraded more rapidly at sites with lower calcium concentrations, resulting in a lower number of recently dead unionids collected overall. Shell degradation is generally related to site factors such as water chemistry and hydrology, where decay occurs more quickly at sites with low dissolved calcium and flowing water, in contrast to calm, alkaline waters (Strayer & Malcom, 2007b). However, significant shell degradation has been reported to occur at sites where calcium is below 15 mg/L (Strayer & Malcom, 2007b), which is below any concentration measured at sites within Lake Memphrémagog. Small and thinner-shelled species tend to exhibit higher decay rates in general (Strayer & Malcom, 2007b; Illari *et al.*, 2015). Therefore, the underrepresentation of *E. complanata* among dead shells is more likely the result of factors that do not assume static conditions at sites, such as burial and fluvial transport offsite (personal observations).

Variation in unionid abundance and biofouling across sites and waterbodies

Sites within Lake Memphrémagog displayed significant spatial variation in unionid density and biofouling, consistent with a previous study that reported heterogeneity in environmental factors (e.g., substrate size, calcium concentration) that influence unionid and zebra mussel distribution in the lake (D'Avignon et al., 2020). Sites did not display any significant variation in recent unionid mortality in the first year of summer sampling; however, mortality varied significantly in the second year. In 2023, the occurrence of recent dead shells was consistently low across sites, whereas empty shells were much more abundant and variable across sites in 2024.

Fouling intensity, mass ratio and recent unionid mortality all varied significantly among Lake Memphrémagog, the Magog River and Lake Magog. In contrast, unionid density did not vary significantly across these three connected waterbodies, possibly as a consequence of differential mortality from previous years. Across all sites in 2024, zebra mussel fouling was likely limited by propagule pressure. Sites with the highest number of attached zebra mussels were located the northern basin of Lake Memphrémagog—the reported initial area of invasion (specifically, Baie de Magog; Picard & Doyon, 2018). The exception is Plage Deauville, which despite being located in Lake Magog, also had a high number of attached zebra mussels.

Biofouling as a function of calcium concentration

Calcium concentration is a limiting factor for zebra mussel distribution and abundance (Ramcharan *et al.,* 1992; Mellina & Rasmussen, 1994; Hincks & Mackie, 1997; Jones & Ricciardi, 2005), and consequently, fouling impacts on unionids (Jokela & Ricciardi, 2008). Zebra mussels were found attached to unionids at all sites except for Fitch Bay, located near the south basin of Lake Memphrémagog, which was completely devoid of zebra mussels. Fitch Bay had the lowest average calcium concentration of all sites sampled ([Ca^{2+}] = 17.4 mg/L), and near the putative threshold for establishment (~15 mg/L; Mellina and Rasmussen, 1994), suggesting a causal link to the absence of zebra mussels. However, sites in close proximity to the outlet of Fitch Bay in the south basin of the lake (i.e., Île Ronde and Cedarville) had mean calcium concentrations ranging from 21.6 to 25.0 mg/L, and still very low abundances of zebra mussels fouling on unionids; whereas sites in the northern basin that had similar calcium concentrations (20.2 to 26.6 mg/L) supported the highest zebra mussel fouling. We found that variation in zebra mussel fouling intensity across sites is not correlated to calcium concentration over the range observed in this system.

Nevertheless, the relationship between mean fouling intensity and calcium concentration in Lake Memphrémagog in 2023 and 2024 fits an empirical model of invaded lakes and rivers in North American and Europe (Jokela & Ricciardi, 2008), although the fit could deviate as biofouling continues to increase. The relationship between unionid mortality and mean mass ratio in Lake Memphrémagog is significantly different from other invaded waterbodies in North America (Ricciardi, 2003), which have calcium concentrations ≥ 30 mg/L and supported chronic zebra mussel densities of at least 1000/m^2 (e.g., Lake St. Clair, Gillis & Mackie, 1994; Detroit River, Ohnesorg *et al*., 1993; Lake Erie, Masteller & Schloesser, 1992; upper St. Lawrence River, Ricciardi *et al*., 1996). Unionid populations in these habitats tended to undergo severe declines to near extirpation within 3-6 years of invasion (Strayer & Malcolm, 2007). The onset of significant unionid mortality in Lake Memphrémagog occurred approximately 7 years following the establishment of zebra mussels, having reached fouling levels that are typically associated with concomitant major population declines in other invaded systems; for example, Baie

Cummins in attained a maximum mean fouling intensity of 91 zebra mussels/m^2 in Summer 2024 and the Sherbrooke Water Intake site in Spring 2024 had a maximum mean mass ratio of 1.47 g zebra mussels/g unionid. We hypothesize that suboptimal calcium concentrations in the lake has slowed the rate of biofouling and thus prolonged the onset of unionid mortality compared with calcium-rich systems (Ricciardi, 2003).

Unionid abundance and biofouling across years and seasons

In Lake Memphrémagog, unionids experienced significantly higher mortality in the summer of 2024 compared to 2023, but this was not reflected in overall mean density estimates. Unionid distribution patterns vary due to, *inter alia*, environmental factors, habitat preferences, and biological factors such as those that enhance reproductive success, including host fish distribution (Vaughn & Taylor, 2000; Strayer, 2008; Cyr *et al*., 2012; Schwalb *et al.,* 2013; Lopez & Vaughn, 2021; Vaessen *et al*., 2024). Thus, spatial habitat heterogeneity and unionid patchiness can lead to substantive differences in unionid density estimates (Downing & Downing, 1992; Hopper *et al.,* 2024). However, significant declines in mean unionid densities were observed at some individual sites between years during the summer, suggesting that lake-wide population declines may become detectable over longer timescales, which is a prevalent challenge when researching long-lived organisms like freshwater mussels (Dodds *et al.,* 2012; Hopper *et al.,* 2024).

Unionid mortality was correlated with the mass ratio of attached zebra mussels to their unionid hosts in Lake Memphrémagog. The relationship between zebra mussel fouling and unionid mortality in North American waterbodies is well-documented (Ricciardi *et al*., 1996; Ricciardi 2003), as are rapid declines of unionid populations following zebra mussel invasion (e.g., Gillis & Mackie, 1994; Ricciardi *et al*., 1996; Strayer *et al*., 1998; Hallac & Marsden, 2000; Martel *et al.,* 2001). Various causal mechanisms have been identified for the increased mortality of unionids due to biofouling, including the hindrance of normal metabolic activities—such as feeding and respiration, due to smothered siphons (Haag *et al*., 1993; Baker & Hornbach, 1997)—as well as the interference with burrowing activity and locomotion (Gillis & Mackie, 1994; Nalepa & Schloesser, 1994). Heavy zebra mussel biofouling can reduce the glycogen, lipid and tissue caloric content in unionids (Hebert *et al.,* 1991; Haag *et al.,* 1993; Baker & Hornbach, 1997; Patterson *et al.,* 1997; Hallac & Marsden, 2000).

It has been previously hypothesized that in north temperate habitats, winter would be the period in which unionid mortality would be greatest following heavy zebra mussel biofouling in the previous year, owing to the inability of unionids to accumulate sufficient energy stores for overwintering (Ricciardi *et al*., 1996). Supporting this hypothesis, Ricciardi et al. (1996) found an ~80% reduction in the density of infested unionids in the upper St. Lawrence River over the winter of 1993-1994 and near total extirpation in the subsequent winter, following 2-3 years of intense fouling; unionids had experienced their lowest caloric values prior to the onset of the winter that preceded their near total extirpation at multiple sites. Other studies reported similar results at infested sites in western Lake Erie (Schloesser & Nalepa, 1994 and Lake St. Clair, observing the greatest number of freshly killed unionids in early spring (Nalepa, 1994). However, in the present study, there was no relationship between zebra mussel biofouling in the fall and unionid mortality in the subsequent spring for two successive years, suggesting that overwintering stress might not be the principal cause of unionid mortality in Lake Memphrémagog.

Another, perhaps larger, source of stress is the energy demand associated with spawning. As species with long lifespans and indeterminate growth, unionids continuously face trade-offs in energy allocation between reproduction, growth, and maintenance (Stearns, 1992). During times of stress, most of their energy is directed toward sustaining basal metabolism, leaving minimal energy for growth and reproduction (Ganser *et al*., 2015). As such, environmental stressors have the potential to disrupt reproductive success in unionids. For example, multiple studies have measured negative effects at temperatures outside of the optimal range of unionids including *E. complanata* (Galbraith & Vaughn, 2009; Ganser *et al.,* 2015; Blakeslee & Lellis, 2019), as their spawning and gamete development are closely linked to thermal conditions. In northeastern North America, spawning and glochidia release for tachytictic species such as *E. complanata* generally occurs in the summer (Downing *et al.,* 1993), raising the possibility that the reproduction of unionids in north temperate lakes could potentially be affected by synergistic effects of climate warming (periods of heat stress) and biofouling.

Is there a unionid refugium in Lake Memphrémagog?

The absence of zebra mussels in the Fitch Bay area could perhaps be attributed to the narrowing of the bay from the outlet of Lake Memphrémagog with dense vegetation acting as a barrier to veliger dispersal (cf. Bodamer & Bossenbroek, 2008). Nearshore areas with similar habitat characteristics to Fitch Bay, such as wetlands and marshy bays with silt-clay sediments and shallow depths (<1 m), have been found to act as refugia in some areas of the Great Lakes (Nichols & Amberg, 1999; Zanatta *et al*., 2002; Bossenbroek *et al*., 2018). Given that the reported initial site of invasion was Baie de Magog in the north basin (Picard & Doyon, 2018), and that the direction of water flow is south to north (Larocque & Dubois, 2012), current zebra mussel distribution in the lake may still be governed by propagule pressure and hydrological patterns, consistent with observations earlier in the invasion (D'Avignon *et al*., 2020). Fouling was more prevalent in Magog Lake than in the Magog River, likely due to higher flow velocities in the river that limit larval settlement on unionids (Claudie & Mackie, 1994; O'Neill, 1996). With the exception of Fitch Bay, unionid populations in the south basin of Lake Memphrémagog (i.e., Île Ronde and Cedarville) were the most abundant within the entire system (Île Ronde in Summer 2024; maximum mean density = 50.4 unionids/m^2) and fouling was uniformly low at these sites. Future surveys are required to determine whether unionid assemblages at these sites remain relatively unfouled (i.e., occupying a refugium) or, alternatively, are in a transient state of lagging zebra mussel colonization (Hopper *et al*., 2023).

Conclusions

The zebra mussel has now caused significant fouling and observed mortality in unionid populations in Lake Memphrémagog, approximately 7 years after invasion began. The lake and connected waters have a mean calcium concentration of ~20 mg/L, which is thought to be suboptimal for sustaining dense zebra mussel populations such as those observed in calcium-rich systems (Cohen & Weinstein, 2001; Therriault, *et al*., 2013). Water chemistry suggests a rationale for the delayed onset of unionid mortality compared to calcium-rich systems, where unionid populations have experienced severe declines and local extirpations within 3-6 years post-invasion. However, fouling intensity is not correlated with calcium concentration in this system, and thus propagule pressure and hydrodynamics likely also play a role. I hypothesize that the unionid community will continue a trajectory towards an assemblage dominated by *Elliptio complanata*, as this species is already the more abundant and apparently less sensitive to fouling than the other unionid species. Rapid unionid mortality is expected to occur in the coming years where there is heavy colonization, as unionids in these areas reach documented thresholds of fouling tolerance. Zebra mussels are therefore capable of exerting significant impacts on native mussels under a calcium regime deemed by environmental risk assessments to be suboptimal. This case underscores the need for risk assessment to provide more nuanced consideration of how the impacts of zebra mussels on native biodiversity vary with water chemistry and other habitat conditions, particularly in heterogenous systems.

Tables

Table 2.1. Physicochemical variables for sites in Lake Memphrémagog averaged (± SD) over: A) 2022-2023 for Fall and 2023-2024 for Spring, and B) 2023-2024 for Summer. If no SD, value taken from most recent year sampled. C) Physicochemical variables for sites in Lake Magog and the Magog River.

A.

Site	Temp. (°C)	pH	Cond. (k Ω cm)	TDS (g/L)	[Ca^{2+}] (mg/L)	Depth (m)	%SAV	MPS (phi)
Île Lord	17.60 ± 2.26	7.93	6.72 ± 0.26	0.094	18.40 ± 1.13	2.40	0	-3.979
Sherbrooke	17.25 ± 1.20	7.83	6.71 ± 0.30	0.094	16.00	6.00	15	1.336
Baie de Magog	15.35 ± 2.05	9.12 ± 3.91	6.15	0.106	17.00 ± 0.28	1.00	0	-1.322
Île Lord	20.20 ± 2.69	8.04 ± 1.89	6.94 ± 0.007	0.094 ± 0.0002	19.40 ± 1.98	2.40	0	-3.979
Sherbrooke	20.80 ± 1.27	8.25 ± 2.90	6.81 ± 0.13	0.095 ± 0.001	20.80 ± 2.26	6.00	15	1.336
Baie de Magog	16.40 ± 5.37	8.58	6.78 ± 0.08	0.100 ± 0.008	19.20 ± 2.26	1.00	0	-1.322

B.

Site	Temp. (°C)	pH	Cond. (k Ω cm)	TDS (g/L)	[Ca^{2+}] (mg/L)	Depth (m)	%SAV	MPS (phi)	Turb. (NTU)	[Mg^{2+}] (mg/L)	[Chl-α] (ug/L)
Rue des Manoirs	23.05 ± 2.19	8.34	6.85	0.098	20.20 ± 3.68	1.00	50	-4.644	0.86	0	-0.219
Baie de l'Anse	24.30 ± 1.84	8.28	6.90	0.094	23.30	1.00	50	2.00	1.25	0.24	-0.408
Baie Cummins	22.90 ± 1.84	8.27	6.89	0.093	23.00 ± 3.68	1.00	10	-1.322	0.58	3.12	-0.144
Quai Knowlton	22.95 ± 3.18	8.93	7.21	0.091	20.60 ± 0.28	3.00	0	-6.305	0.57	0.96	-4.747
Pointe Cummins	23.95 ± 1.77	8.69	7.05	0.092	24.20 ± 4.24	5.00	0	-5.474	0.29	1.92	0.108
Île à l'Aigle	23.60 ± 1.41	8.62	6.90	0.094	26.60 ± 7.64	4.00	50	-2.983	0.34	0.48	-0.488
Rue Colby	24.20 ± 0.28	8.69	7.31	0.089	25.00 ± 5.37	2.50	50	2.00	0.61	0.48	0.039
Penfield	23.75 ± 0.07	8.92	7.31	0.089	22.80 ± 1.70	1.00	0	-1.322	0.94	1.44	-0.114
Fitch Bay	23.50 ± 1.56	8.71	9.10	0.071	17.40 ± 1.98	1.00	75	2.00	2.85	1.92	0.592
Cedarville	22.20	NA	NA	NA	21.60	1.00	20	-1.322	0.91	0.24	1.638
Île Ronde	24.40	NA	NA	NA	22.00	2.50	5	-6.305	0.95	0	0.488

Temp. = temperature; Cond. = conductivity; TDS = total dissolved solids; [Ca^{2+}] = calcium concentration, %SAV = submerged aquatic vegetation; MPS = mean particle size of sediment; Turb. = turbidity; [Mg^{2+}] = magnesium concentration; Chl-α = chlorophyll-α concentration

Table 2.1. (continued) Physicochemical variables for sites in Lake Memphrémagog averaged (± SD) over: A) 2022-2023 for Fall and 2023-2024 for Spring, and B) 2023-2024 for Summer. If no SD, value taken from most recent year sampled. C) Physicochemical variables for sites in Lake Magog and the Magog River.

C.

Site	Temp. (°C)	[Ca^{2+}] (mg/L)	Depth (m)	%SAV	MPS (phi)	Turb. (NTU)	[Mg^{2+}] (mg/L)	[Chl-α] (ug/L)
Rue des Colombes	25.50	20.00	0.5	35	-2.152	1.48	1.92	-0.159
Plage Deauville	23.70	22.80	1.00	15	-7.966	0.85	0.72	-0.604
Club Nautique	24.90	19.20	0.50	10	-3.979	0.79	1.92	-0.197
Descente Bournival	24.70	21.20	2.00	10	2.00	1.32	0.72	-0.664
Halte Turgeon-Gaudrault	23.10	20.40	1.00	40	-7.301	0.55	0.96	-0.816

Temp. = temperature; [Ca^{2+}] = calcium concentration, %SAV = submerged aquatic vegetation; MPS = mean particle size of sediment; Turb. = turbidity; [Mg^{2+}] = magnesium concentration; Chl-α = chlorophyll-α concentration

Table 2.2. Summary of mean unionid densities (± SEM) and recent unionid mortality (%) for sites sampled in Summer 2023 & 2024 in Lake Memphrémagog, Magog River and Lake Magog.

Site	Summer 2023		Summer 2024	
	Mean unionid density/m^2	% Recent unionid mortality	Mean unionid density/m^2	% Recent unionid mortality
Lake Memphrémagog				
Rue des Manoirs	2.70 ± 0.52	0	1.10 ± 0.41	26.67
Baie de l'Anse	4.10 ± 0.95	4.88	5.60 ± 0.51	3.45
Baie Cummins	2.80 ± 0.57	6.90	2.30 ± 0.56	8.70
$^\beta$Quai Knowlton	2.30 ± 0.43	8.33	1.30 ± 0.40	12.50
Pointe Cummins	28.60 ± 10.19	1.40	5.00 ± 0.83	8.33
Île à l'Aigle	21.40 ± 6.73	5.61	21.60 ± 7.09	18.18
Rue Colby	13.40 ± 0.93	0	20.60 ± 10.38	18.18
Penfield	5.80 ± 0.83	0	7.40 ± 1.36	5.13
Lake Memphrémagog – south basin				
$^\alpha$Fitch Bay			1.50 ± 0.73	6.25
Cedarville			35.60 ± 3.36	2.20
Île Ronde			50.40 ± 7.22	0.38
Magog River				
Halte Turgeon-Gaudrault			4.60 ± 2.68	17.24
Lake Magog				
Rue des Colombes			12.40 ± 4.13	0
Descente Bournival			2.60 ± 0.45	0
Club Nautique			3.30 ± 0.47	5.71
Plage Deauville			23.40 ± 4.43	0.008

$^\alpha$No zebra mussels detected.

Bold = sites where the mean unionid density in 2024 is significantly lower than in 2023, based on paired two-sample t-test ($p < 0.05$); $^\beta p = 0.056$.

Table 2.3. Summary of mean unionid densities (± SEM) and recent unionid mortality (%) for sites sampled every Fall and Spring from 2022-2024 in Lake Memphrémagog.

Site	Fall 2022		Spring 2023		Fall 2023		Spring 2024	
	Mean unionid density /m^2	% Recent unionid mortality	Mean unionid density/ m^2	% Recent unionid mortality	Mean unionid density/ m^2	% Recent unionid mortality	Mean unionid density /m^2	% Recent unionid mortality
Île Lord	13.20 ± 1.24	4.35	6.60 ± 1.29	5.97	8.10 ± 1.32	2.47	7.60 ± 2.91	2.56
Baie de Magog	23.00 ± 4.47	0	24.60 ± 4.86	3.25	37.80 ± 5.98	5.82	30.00 ± 5.50	6.67
Sherbrooke Water Intake	14.40 ± 6.56	5.19	28.80 ± 2.56	2.78	11.60 ± 2.09	13.79	6.40 ± 1.89	43.10

Table 2.4. Summary of biofouling metrics: mean fouling intensity (# zebra mussels/unionid ± SEM) and mean mass ratio (g zebra mussels/g unionid ± SEM) for sites sampled in Summer 2023 & 2024 in Lake Memphrémagog, Magog River and Lake Magog.

Site	Summer 2023		Summer 2024	
	Mean fouling intensity	Mean mass ratio	Mean fouling intensity	Mean mass ratio
Lake Memphrémagog				
Rue des Manoirs	27.11 ± 3.71	0.48 ± 0.07	19.09 ± 4.83	0.99 ± 0.28
Baie de l'Anse	0.68 ± 0.20	0.16 ± 0.08	0.54 ± 0.16	0.08 ± 0.02
Baie Cummins	1.04 ± 0.31	0.09 ± 0.02	91.05 ± 12.15	0.75 ± 0.11
Quai Knowlton	0	0	0.15 ± 0.10	0.33 ± 0.22
Pointe Cummins	2.49 ± 0.45	0.12 ± 0.01	7.92 ± 1.28	0.38 ± 0.05
Île à l'Aigle	19.29 ± 1.60	0.35 ± 0.03	25.18 ± 1.49	0.70 ± 0.04
Rue Colby	0.38 ± 0.09	0.19 ± 0.07	0.50 ± 0.08	0.30 ± 0.05
Penfield	0.05 ± 0.03	0.12 ± 0.04	0	0
Lake Memphrémagog – south basin				
$^{\alpha}$Fitch Bay			0	0
Cedarville			0.11 ± 0.03	0.17 ± 0.03
Île Ronde			0.15 ± 0.03	0.22 ± 0.03
Magog River				
Halte Turgeon-Gaudrault			1.70 ± 0.38	0.04 ± 0.009
Lake Magog				
Rue des Colombes			3.61 ± 0.65	0.26 ± 0.07
Descente Bournival			6.23 ± 1.14	0.11 ± 0.03
Club Nautique			4.21 ± 0.50	0.26 ± 0.04
Plage Deauville			7.75 ± 0.69	0.08 ± 0.008

$^{\alpha}$No zebra mussels detected.
Bold = sites where the mean fouling intensity in 2024 is significantly higher than in 2023, based on Wilcoxon test ($p < 0.05$).

Table 2.5. Summary of biofouling metrics: mean fouling intensity (# zebra mussels/unionid ± SEM) and mean mass ratio (g zebra mussels/g unionid ± SEM) for sites sampled every Fall and Spring from 2022-2024.

Site	Fall 2022		Spring 2023		Fall 2023		Spring 2024	
	Mean fouling intensity	Mean mass ratio	Mean fouling intensity	Mean mass ratio	Mean fouling intensity	Mean mass ratio	Mean fouling intensity	Mean mass ratio
Île Lord	0.36 ± 0.09	0.05 ± 0.02	0.67 ± 0.13	0.18 ± 0.03	1.18 ± 0.14	0.52 ± 0.07	0.77 ± 0.12	0.11 ± 0.01
Baie de Magog	5.70 ± 0.70	0.29 ± 0.05	4.87 ± 0.41	0.42 ± 0.06	9.66 ± 0.56	0.33 ± 0.02	23.70 ± 1.28	0.79 ± 0.07
Sherbrooke Water Intake	25.35 ± 1.92	0.28 ± 0.05	43.04 ± 1.99	0.33 ± 0.02	38.04 ± 3.55	1.67 ± 0.18	37.66 ± 2.47	1.47 ± 0.20

Figures

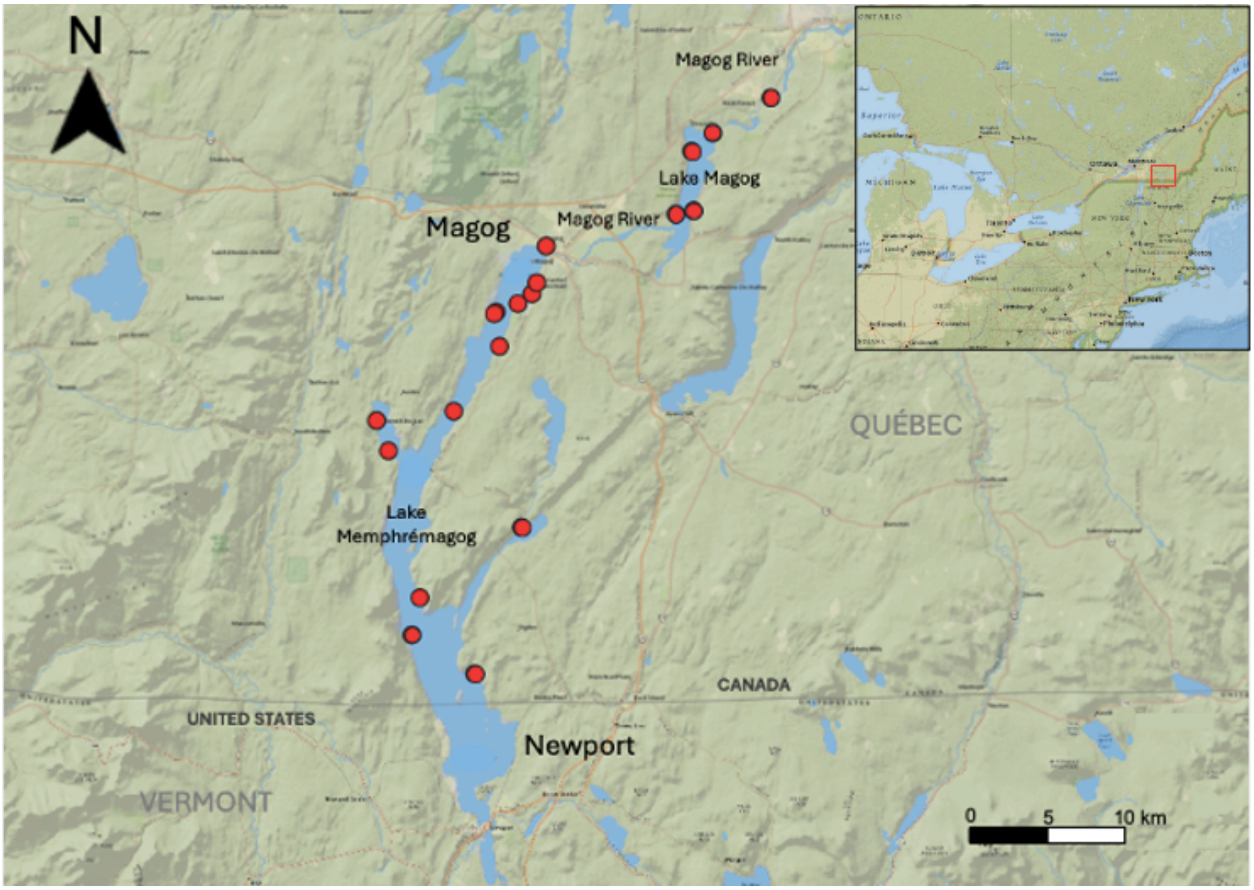


Figure 2.1. Map of sampling sites with inset map of eastern North America. Created with QGIS version 3.32.

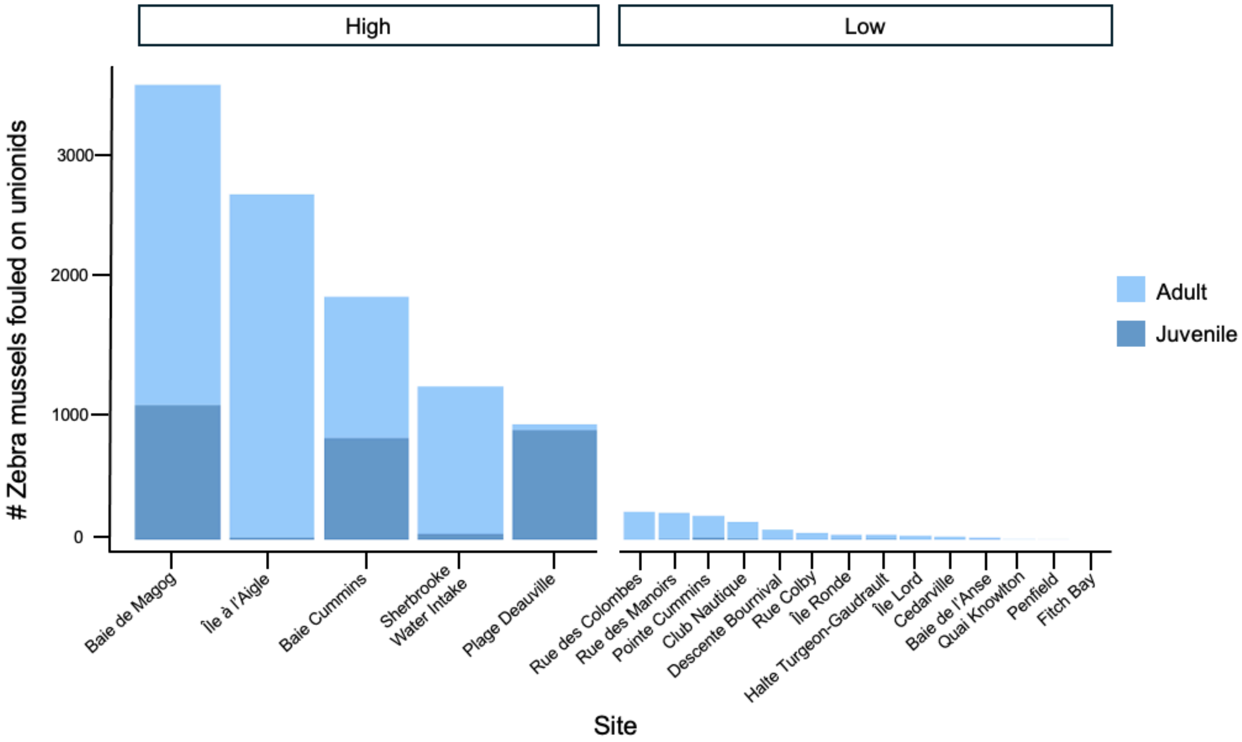


Figure 2.2. Population structure of zebra mussels on live unionids in Lake Memphrémagog, Magog Lake and Magog River in 2024. The number of adult zebra mussels are indicated in light blue (>10 mm), and the number juvenile zebra mussels (<10 mm) are indicated in dark blue. Sites are separated by the total numbers of attached zebra mussels on the unionid assemblages: "High", n>500; "Low", n<500.

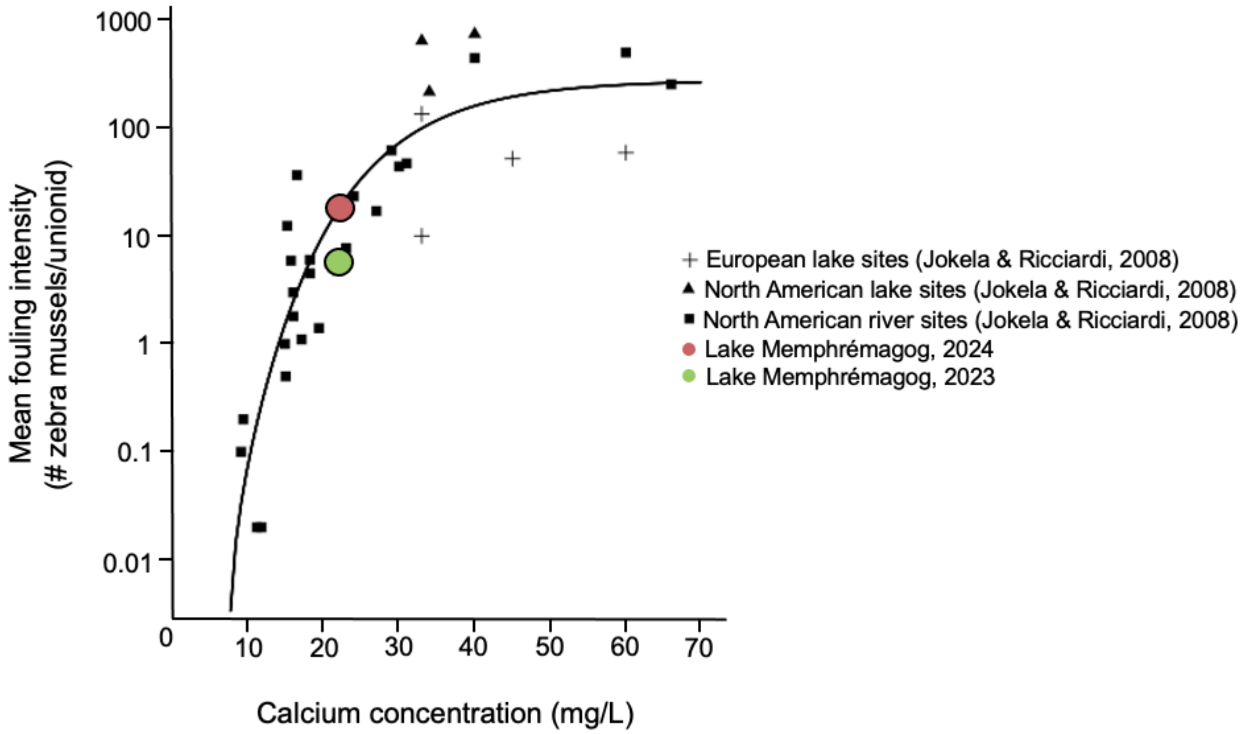


Figure 2.3. Maximum mean fouling intensity as a function of calcium concentration: $\log_{10}(y + 0.01) = -9.19 + 8.63[1 - e^{-0.09x}]$; $R^2 = 0.82$, $p < 0.0001$. Modified from Jokela & Ricciardi (2008) by the addition of 2023 and 2024 data from Lake Memphrémagog.

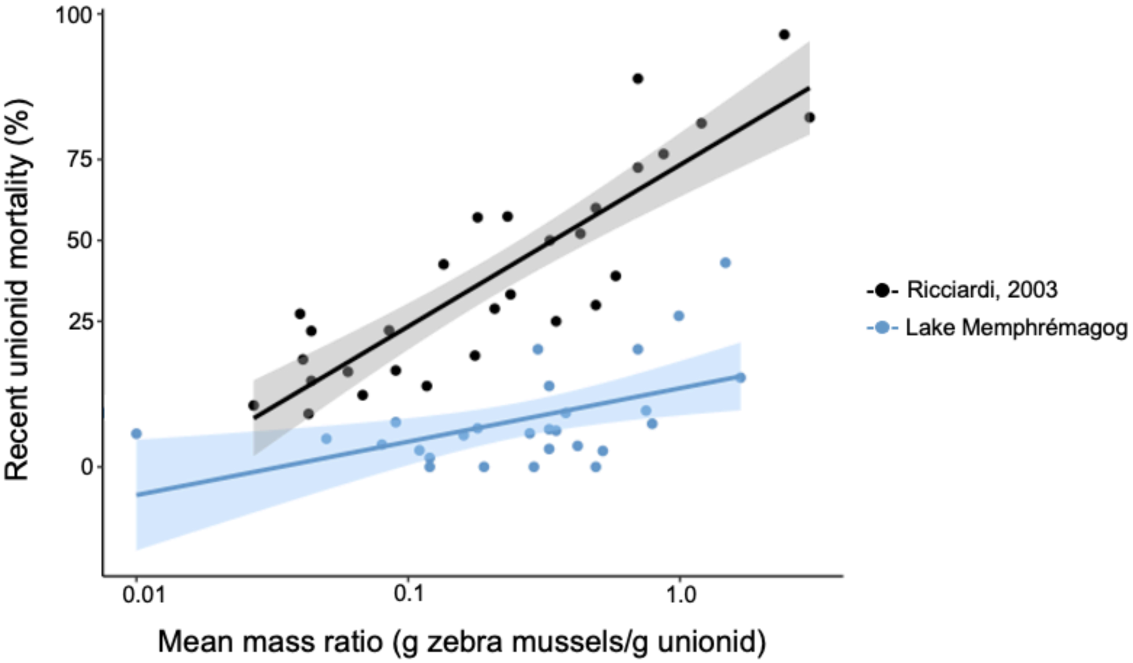


Figure 2.4. Proportion of recently dead unionids as a function of the zebra mussel-unionid mass ratio (wet weights) for North American unionid populations with $[Ca^{2+}] \geq 30$mg/L (black): $\sin^{-1}(y^{0.5}) = 0.48 \log x + 1.0$ ($R^2 = 0.75$, $p < 0.0001$, n = 29) and Lake Memphrémagog (blue): $\sin^{-1}(y^{0.5}) = 2.03 \log x + 4.2$ ($R^2 = 0.49$, $p < 0.0001$, n = 27). Modified from Ricciardi (2003). An ANCOVA revealed a significant difference between regressions (F(1,54) = 65.96, $p < 0.0001$).

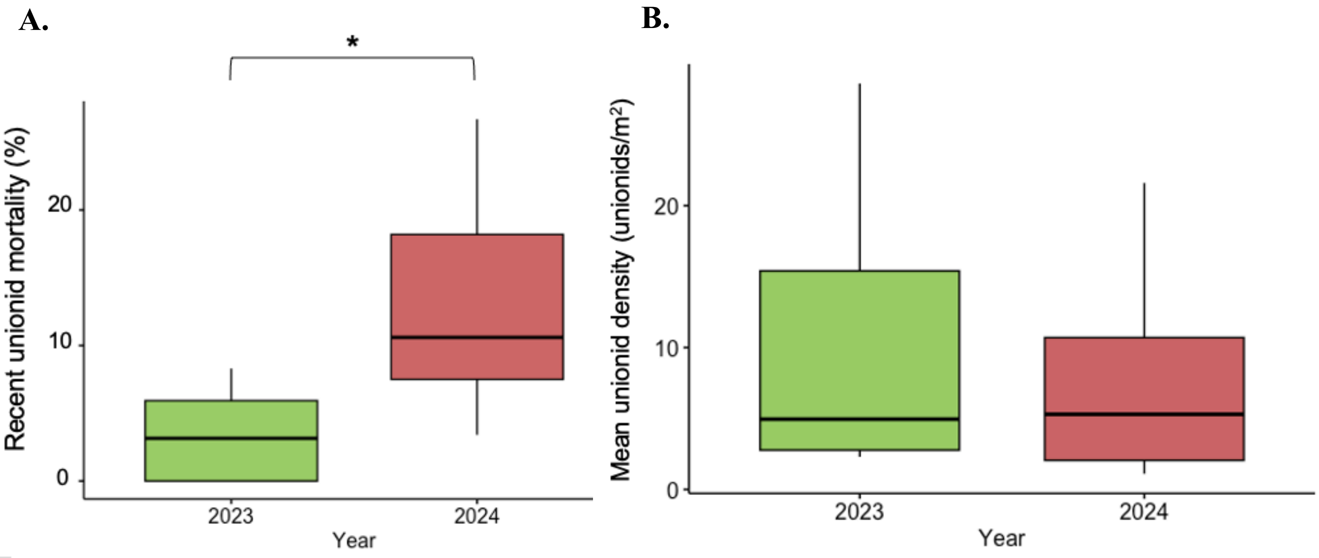


Figure 2.5. A) Recent unionid mortality at 8 sites in Lake Memphrémagog between 2023 and 2024. A paired two-sample t-test revealed a significant increase in recent unionid mortality from 2023 to 2024 (t(7) = -2.744, *p* = 0.01437). B) The mean unionid density (unionids/m^2) at 8 sites between 2023 and 2024. A paired two-sample Wilcoxon test revealed no significant decline in mean unionid density from 2023 to 2024 (V(7) = 18, *p* = 0.5273). * = means differed.

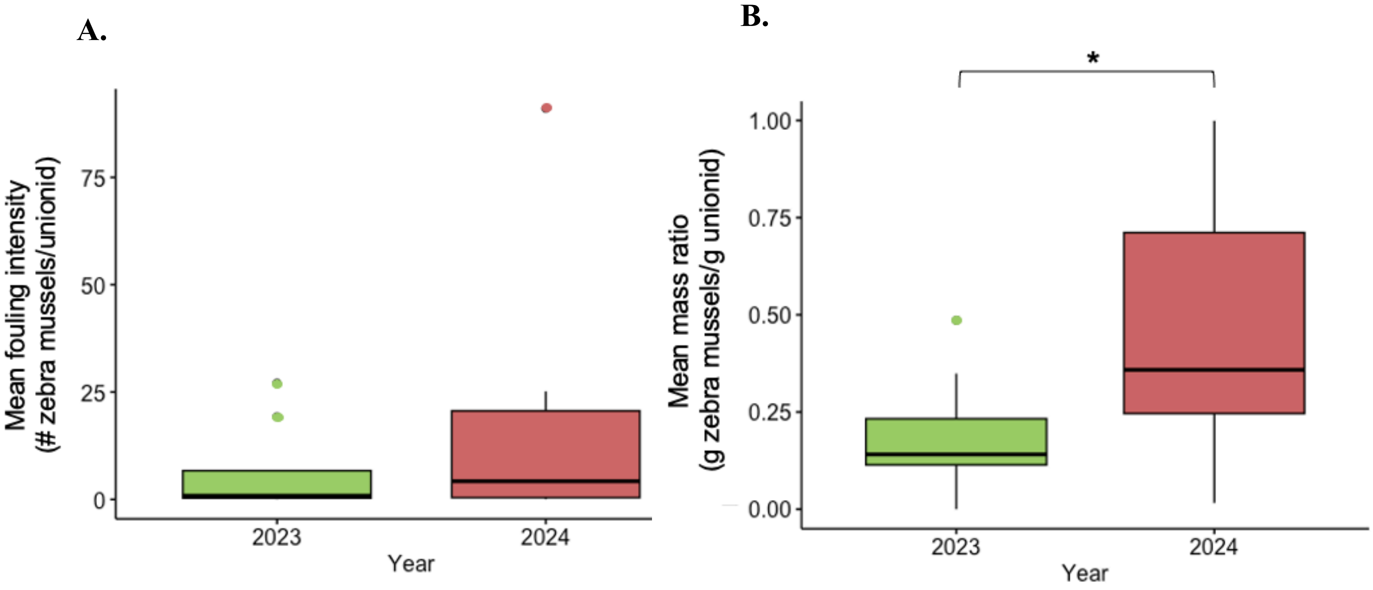


Figure 2.6. A) Mean fouling intensity (# zebra mussels/unionid) at 8 sites between 2023 and 2024. A paired two-sample Wilcoxon test revealed no significant increase in mean fouling intensity from 2023 to 2024 (V(7) = 12, *p* = 0.2305). B) The mean mass ratio (g zebra mussels/g unionid) at 8 sites between 2023 and 2024. A paired two-sample t-test revealed a significant increase in mean mass ratio from 2023 to 2024 (t(7) = -2.6705, *p* = 0.01599).
* = means differed.

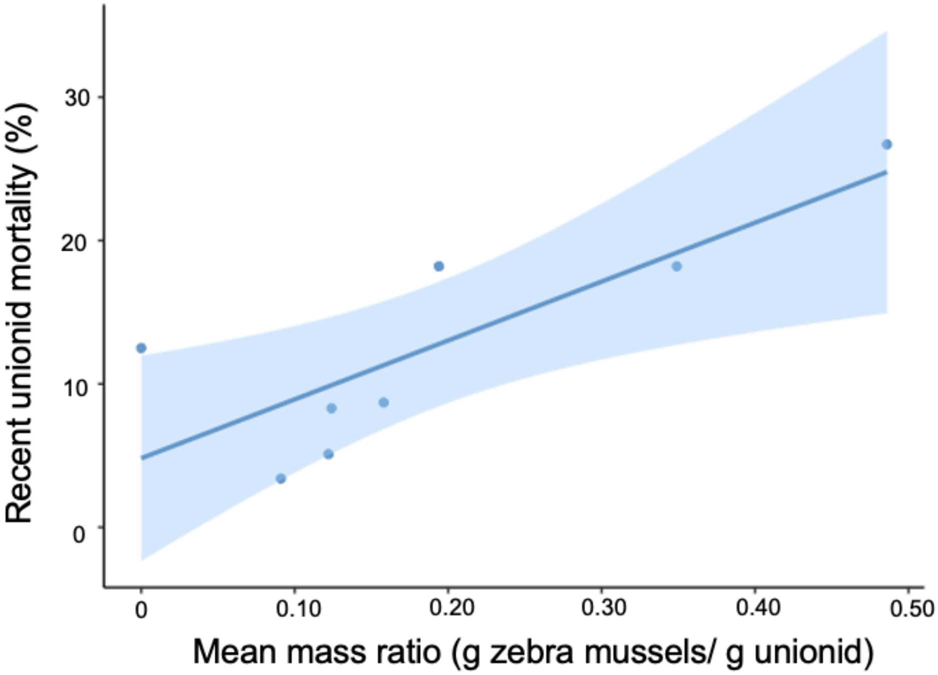


Figure 2.7. The relationship between recent unionid mortality (%) in summer 2024 and the mean mass ratio (g zebra mussels/g unionid) in summer 2023 at 8 sites in Lake Memphrémagog. A simple linear regression analysis ($y = 0.04812 + 0.41078x$) indicated a significant relationship between recent unionid mortality and mean mass ratio (F(1,6) = 11.3 , $R^2 = 0.6532$, $p = 0.0152$).

Supplementary Materials: Tables

Table S2.1. Summary of sampling sites and the dates they were sampled in Lake Memphrémagog, Magog River and Lake Magog.

Site	Water body	Latitude	Longitude	Dates sampled
Île Lord	Lake Memphrémagog	45.1742	-72.2372	Sep-26-2022
				Jun-02-2023
				Oct-13-2023
				Jun-07-2024
Baie de Magog	Lake Memphrémagog	45.2694	-72.1613	Oct-01-2022
				Jun-03-2023
				Oct-13-2023
				Jun-04-2024
Sherbrooke Water Intake	Lake Memphrémagog	45.2484	-72.1690	Sep-26-2022
				Jun-02-2022
				Oct-13-2023
				Jun-07-2024
Rue des Manoirs	Lake Memphrémagog	45.2419	-72.1730	Jul-24-2024
				Jul-04-2024
Baie de l’Anse	Lake Memphrémagog	45.2117	-72.1996	Jul-24-2023
				Jul-04-2024
Île à l’Aigle	Lake Memphrémagog	45.2363	-72.1846	Jul-26-2023
				Jul-06-2024
Pointe Cummins	Lake Memphrémagog	45.2315	-72.2024	Jul-26-2024
				Jul-06-2024
Baie Cummins	Lake Memphrémagog	45.2304	-72.2039	Jul-25-2023
				Jul-02-2024
Quai Knowlton	Lake Memphrémagog	45.1514	-72.2909	Jul-25-2023
				Jul-02-2024
Rue Colby	Lake Memphrémagog	45.0666	-72.2650	Jul-26-2023
				Jul-13-2024
Penfield	Lake Memphrémagog	45.1689	-72.3005	Jul-27-2023
				Jul-08-2024
Fitch Bay	Lake Memphrémagog (south basin)	45.1073	-72.1816	Jul-03-2024
Cedarville	Lake Memphrémagog (south basin)	45.0224	-72.2168	Jul-03-2024
Île Ronde	Lake Memphrémagog (south basin)	45.0442	-72.2699	Jul-13-2024
Halte Turgeon-Gaudrault	Magog River	45.3548	-71.9769	Jul-05-2024
Rue des Colombes	Lake Magog	45.2898	-72.0402	Jul-10-2024
Descente Bournival	Lake Magog	45.2878	-72.0548	Jul-09-2024
Club Nautique	Lake Magog	45.3240	-72.0418	Jul-09-2024
Plage Deauville	Lake Magog	45.3344	-72.0244	Jul-05-2024

Supplementary Materials: Figures

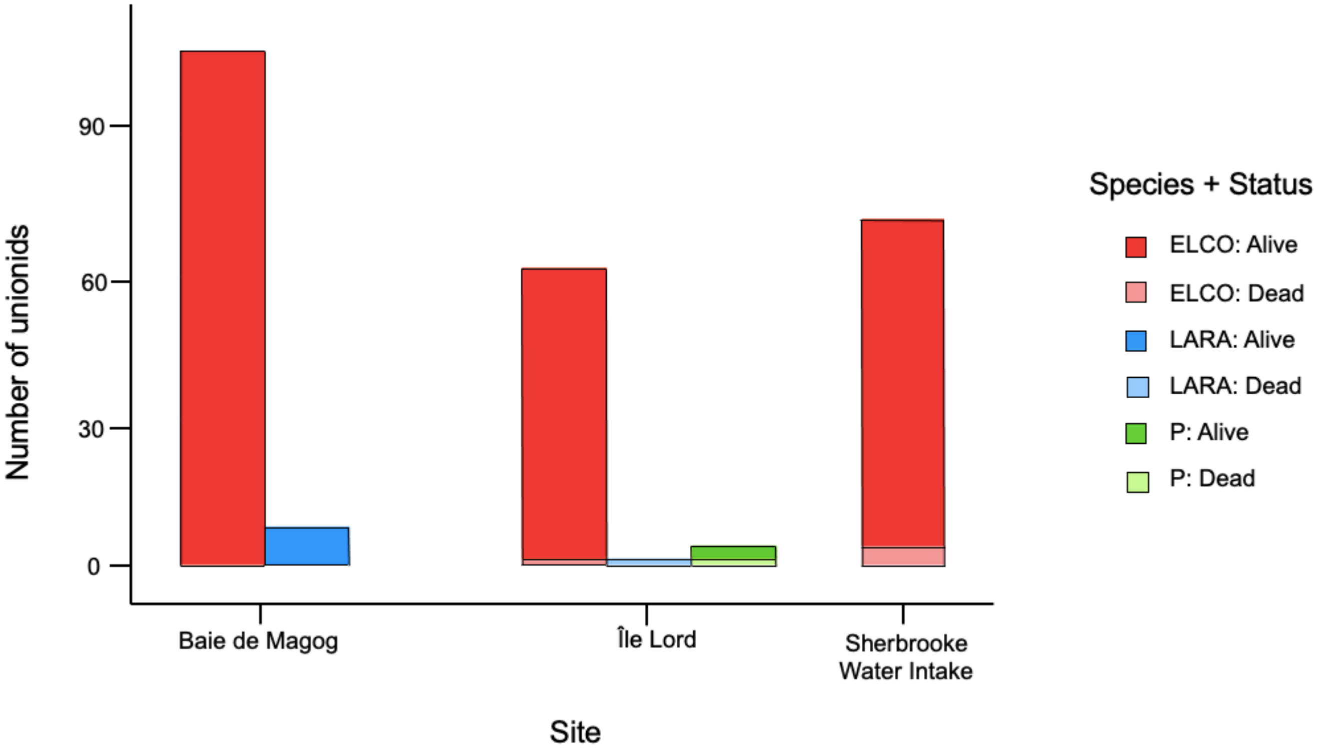


Figure S2.1. The number of unionids collected during Fall sampling at sites in Lake Memphrémagog in 2022 and their status (alive or recently dead). Species codes: ELCO = *Elliptio complanata*, LARA = *Lampsilis radiata*, P = *Pyganodon* sp.

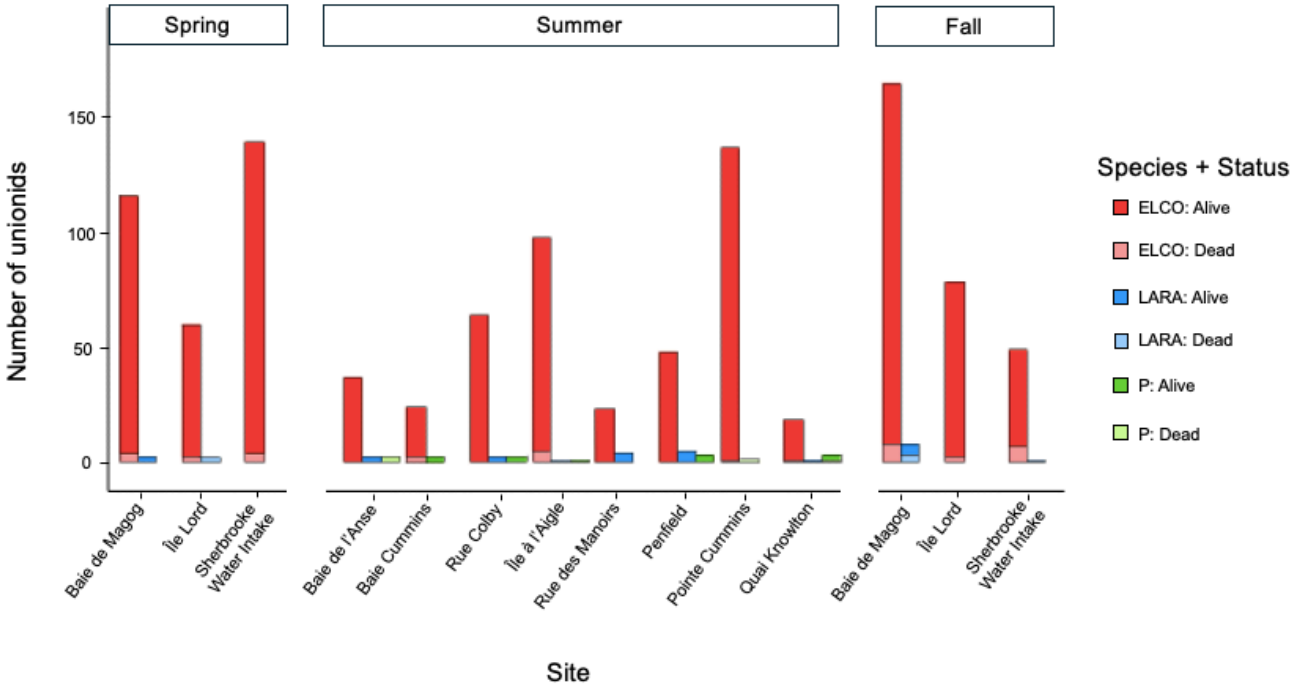


Figure S2.2. The number of unionids collected during Spring, Summer and Fall sampling in Lake Memphrémagog in 2023 and their status (alive or recently dead). Species codes: ELCO = *Elliptio complanata*, LARA = *Lampsilis radiata*, P = *Pyganodon* sp.

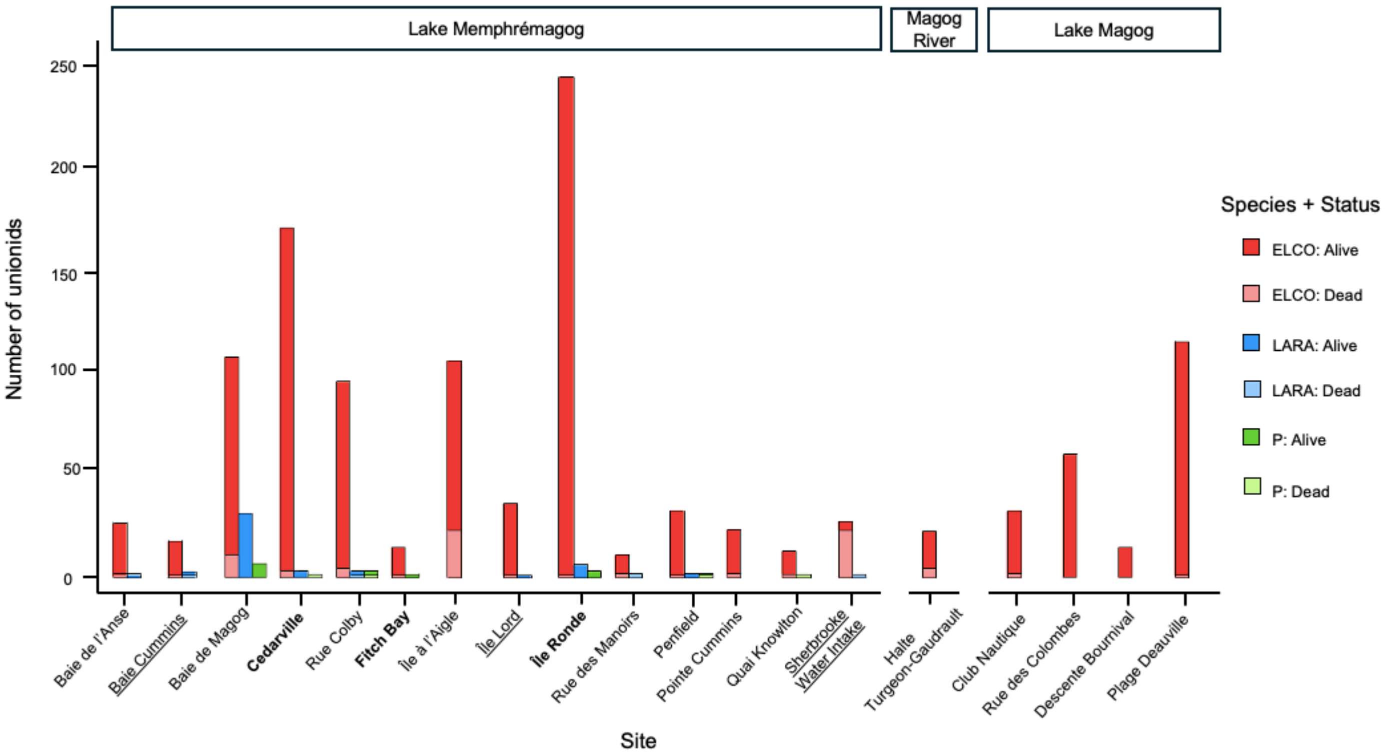


Figure S2.3. The number of unionids collected in summer 2024 in Lake Memphrémagog, the Magog River, and Lake Magog and their status (alive or recently dead). Species codes: ELCO = *Elliptio complanata*, LARA = *Lampsilis radiata*, P = *Pyganodon* sp. Bolded sites in Lake Memphrémagog are found in the southern basin of the lake. Underlined sites in Lake Memphrémagog were sampled in the Spring.

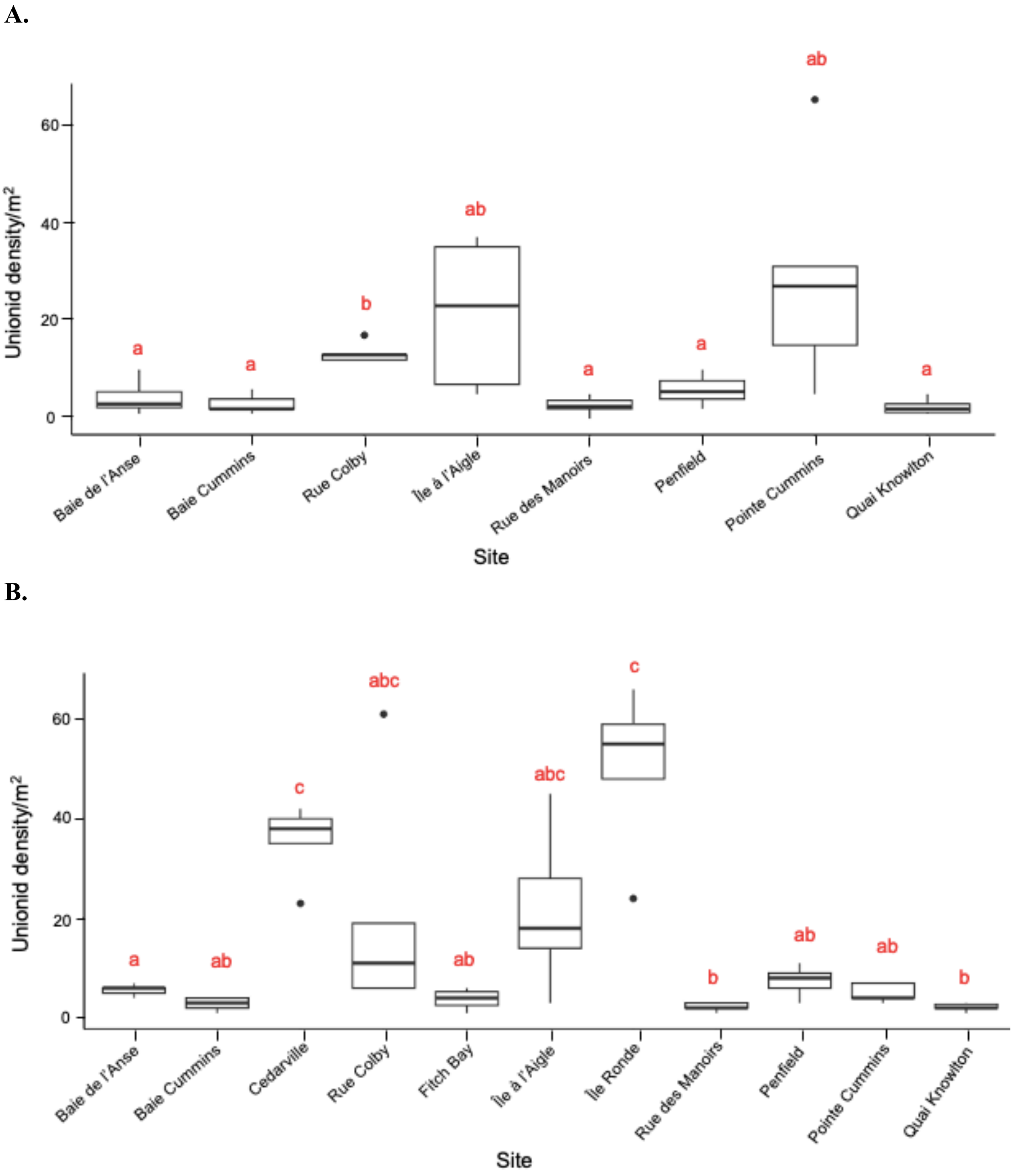


Figure S2.4. The mean unionid density significantly differed across summer sites in Lake Memphrémagog in A) 2023(Kruskal-Wallis: $X^2(7) = 36.351$, $p < 0.0001$) and B) 2024 ($X^2(10) = 43.604$, $p < 0.0001$). Sites with different letters in red represent sites that are significantly different from each other, according to a Games-Howell test.

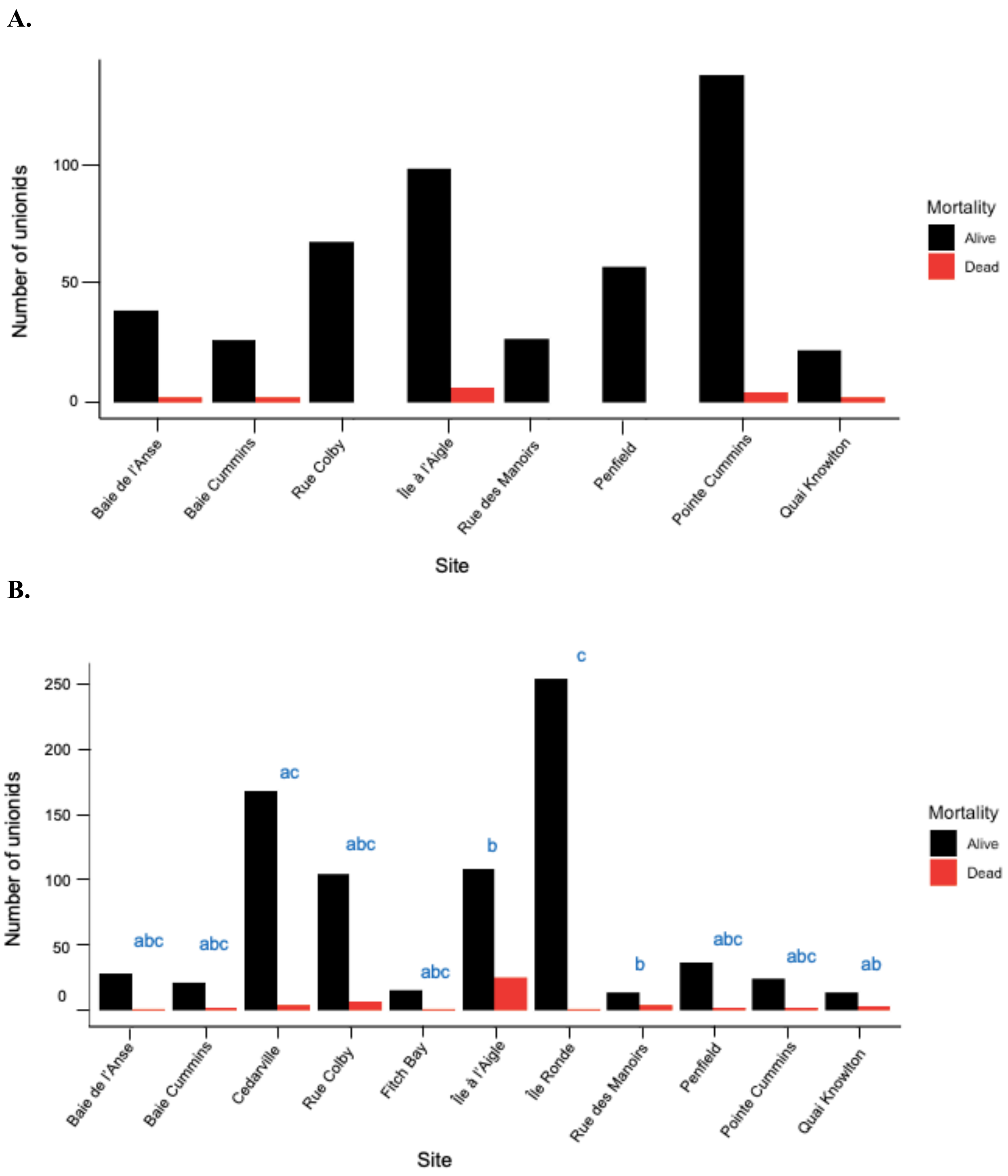


Figure S2.5. The number of unionids across summer sites in Lake Memphrémagog and their status (alive or recently dead). A) The proportion of recently dead unionids did not significantly differ across sites in 2023 (Kruskal-Wallis: $X^2(7) = 10.879$, $p = 0.144$. B) The proportion of recently dead unionids significantly differed across sites in 2024 ($X^2(10) = 69.159$, $p < 0.0001$). Sites with different letters in blue represent sites that are significantly different from each other, according to a Games-Howell test.

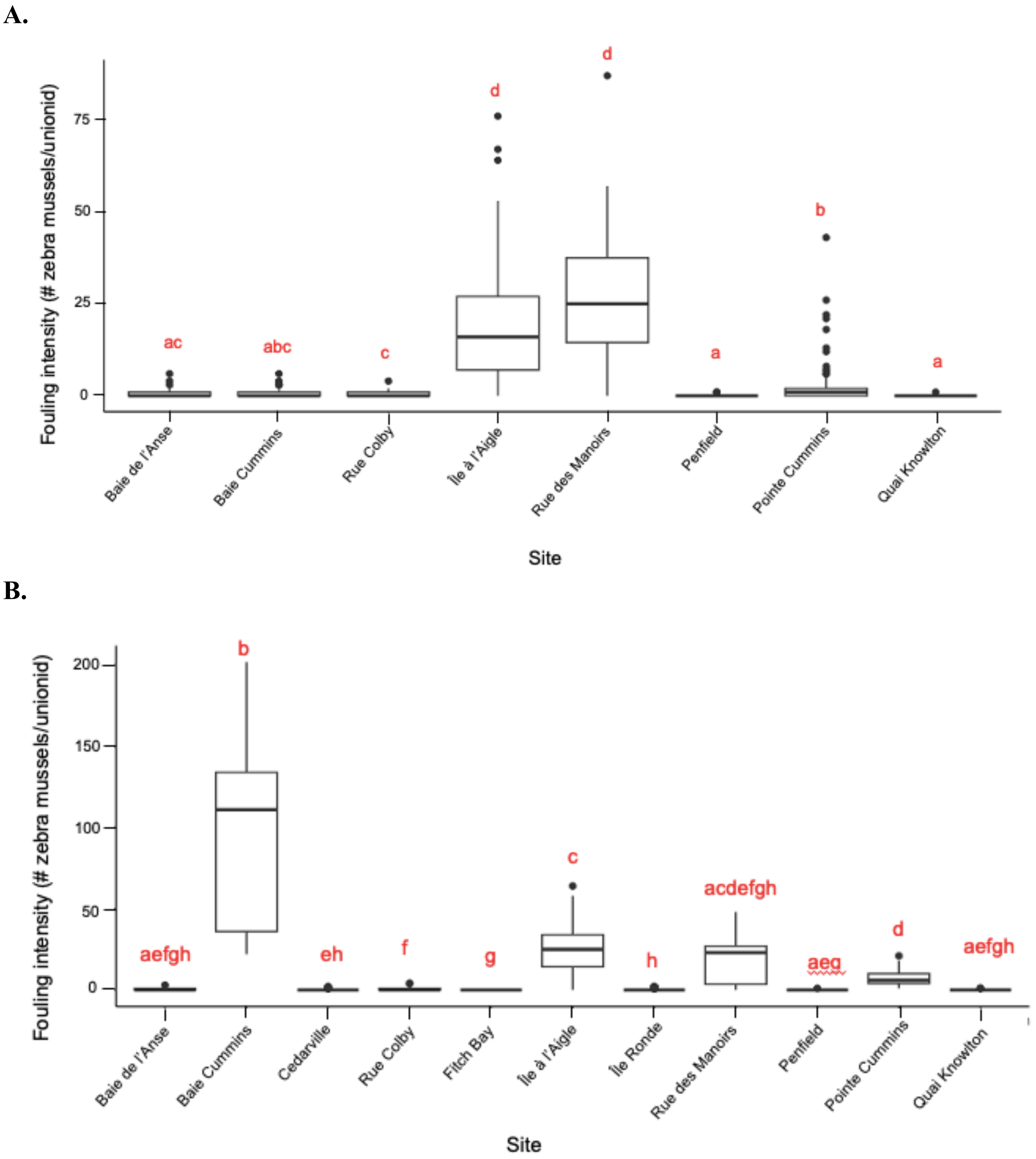


Figure S2.6. The mean fouling intensity significantly differed across summer sites in Lake Memphrémagog in A) 2023 (Kruskal-Wallis: $X^2(7) = 305.36$, $p < 0.0001$) and B) 2024 ($X^2(10) = 551.98$, $p < 0.0001$). Sites with different letters in red represent sites that are significantly different from each other, according to a Games-Howell test.

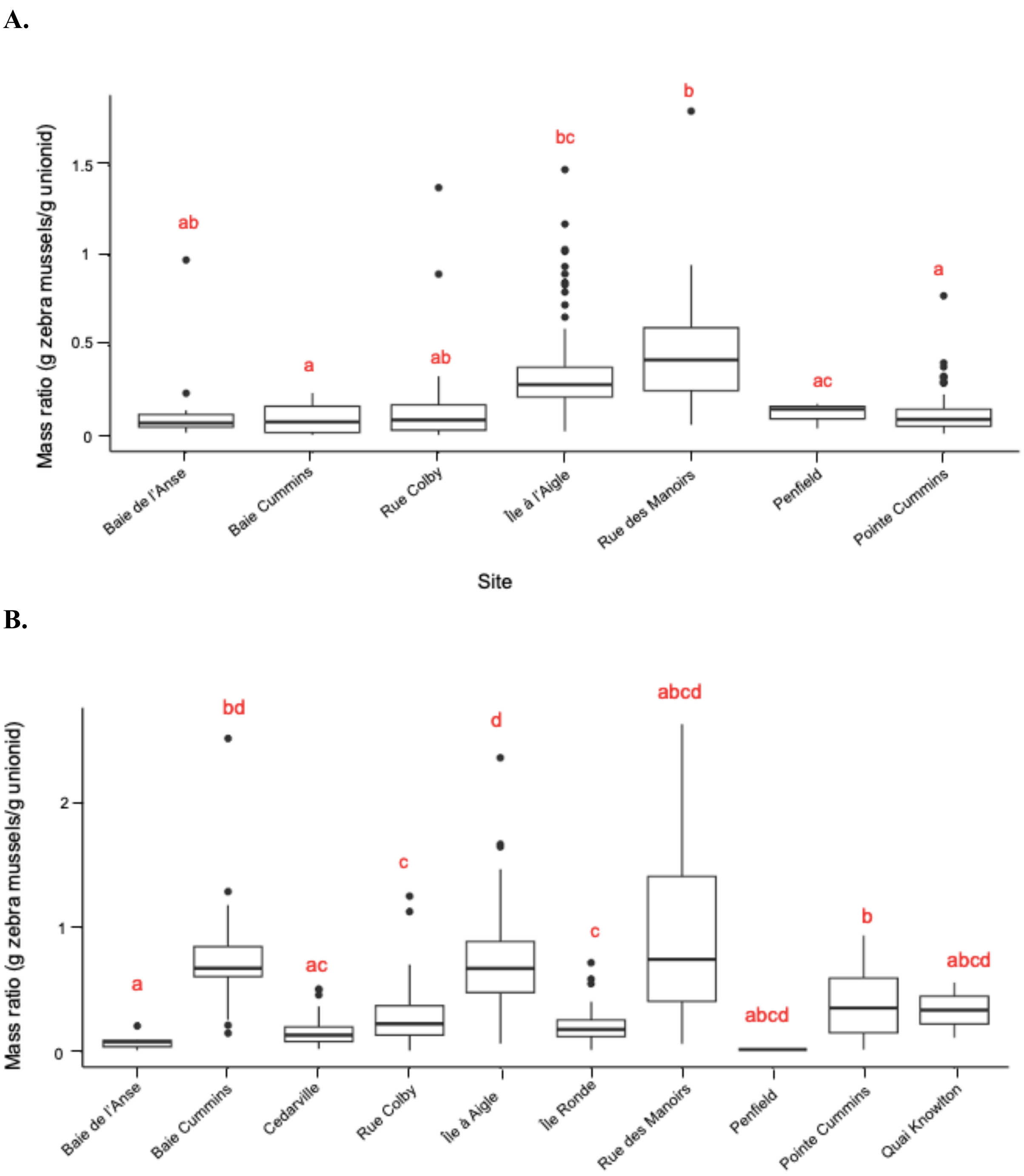


Figure S2.7. The mean mass ratio significantly differed across summer sites in Lake Memphrémagog in A) 2023 (Kruskal-Wallis: $X^2(6) = 105.02$, $p < 0.0001$) and B) 2024 ($X^2(9) = 112.12$, $p < 0.0001$). Sites with different letters in red represent sites that are significantly different from each other, according to a Games-Howell test.

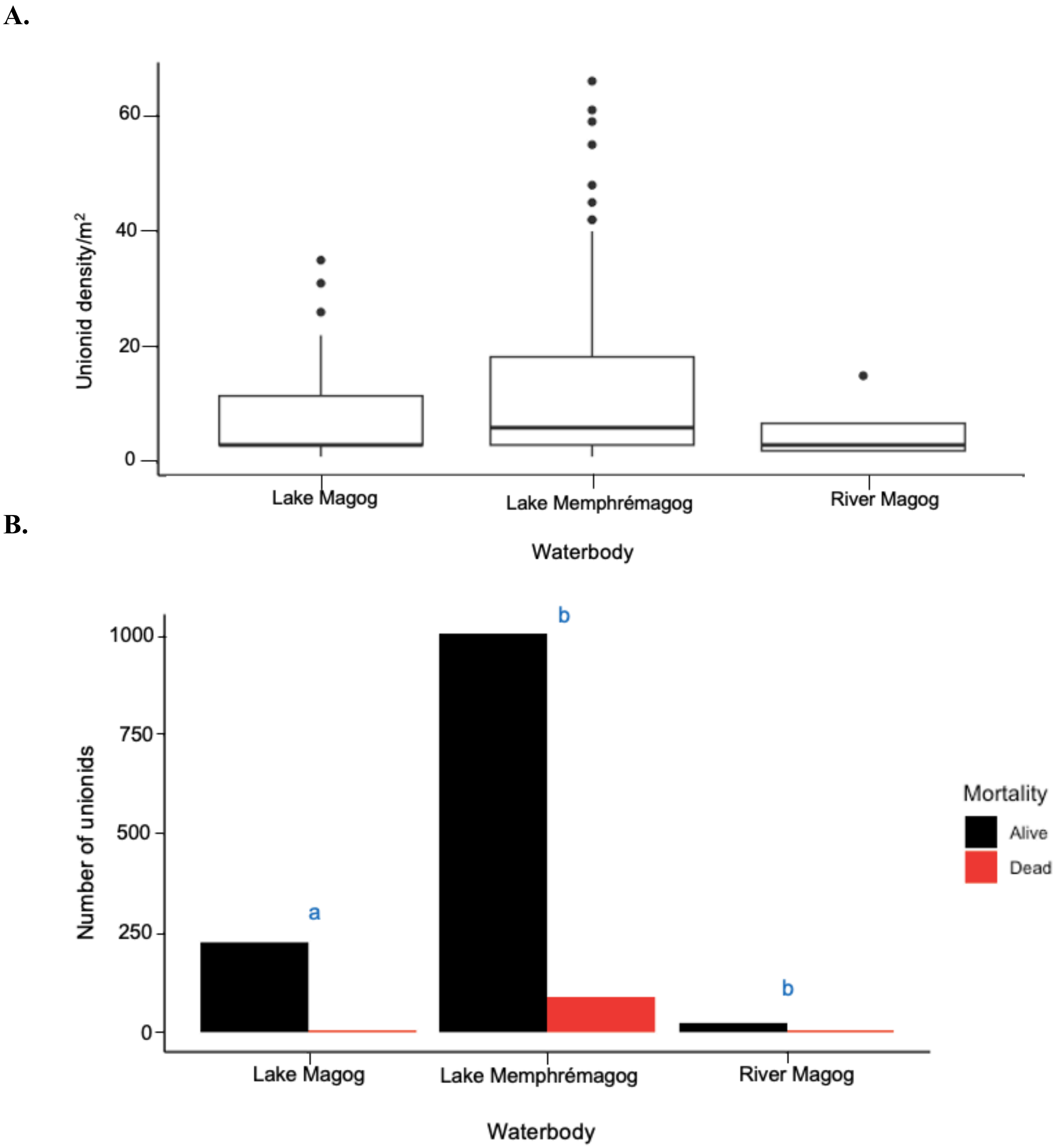
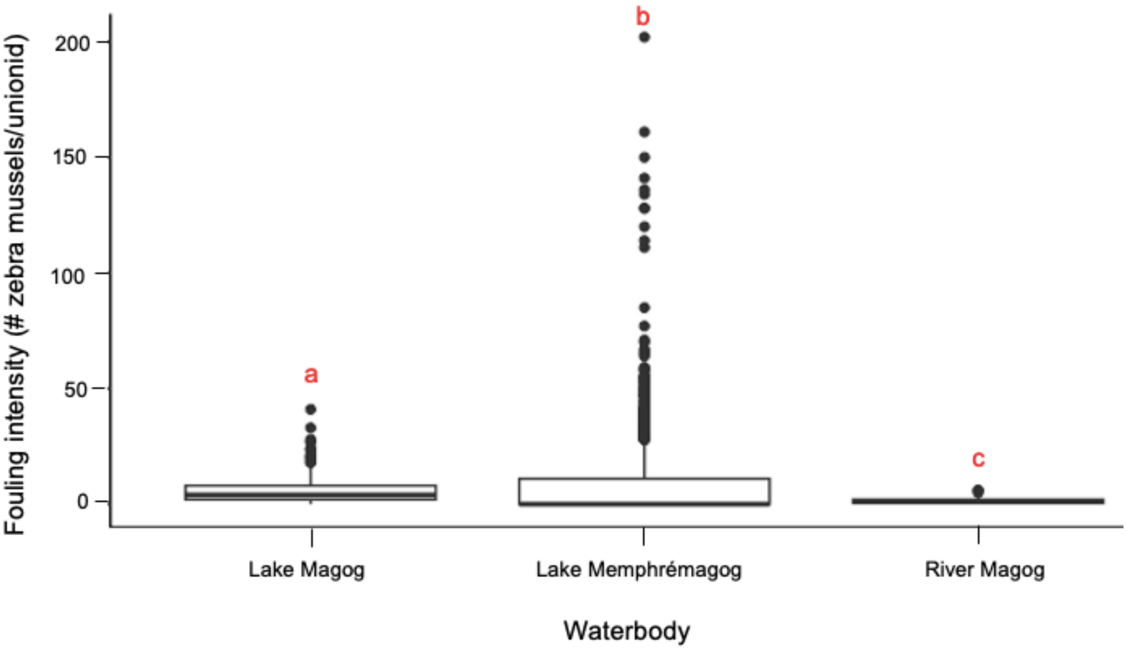


Figure S2.8. A) The mean unionid density/m^2 in Lake Magog, Lake Memphrémagog, and the Magog River in summer 2024, which did not differ significantly across waterbodies (Kruskal-Wallis: $X^2(2) = 3.4185$, $p = 0.181$ B) The proportion of recently dead unionids differed significantly across waterbodies ($X^2(2) = 18.128$, $p < 0.0001$). Sites with different letters in blue represent sites that are significantly different from each other, according to a Games-Howell test.

A.



B.

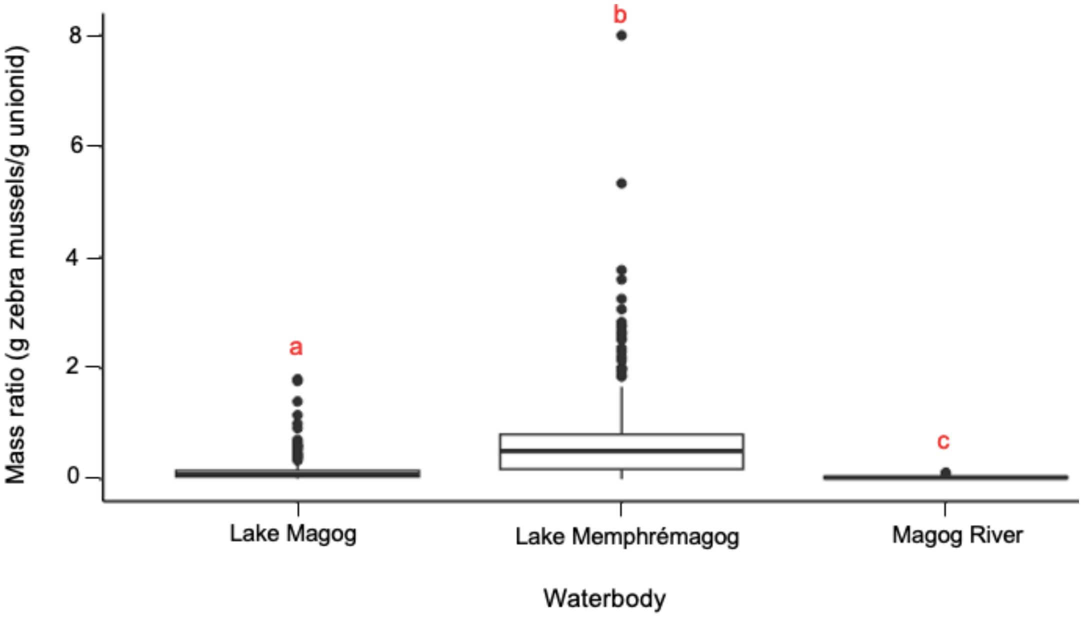


Figure S2.9. Mean biofouling levels in Lake Magog, Lake Memphrémagog and the Magog River in summer 2024. A) The mean fouling intensity differed significantly across waterbodies (Welch's one-way: F(2) = 67.501, $p < 0.0001$). B) The mean mass ratio differed significantly across waterbodies (F(2) = 160.7, $p < 0.0001$). Sites with different letters in red represent sites that are significantly different from each other, according to a Games-Howell test.

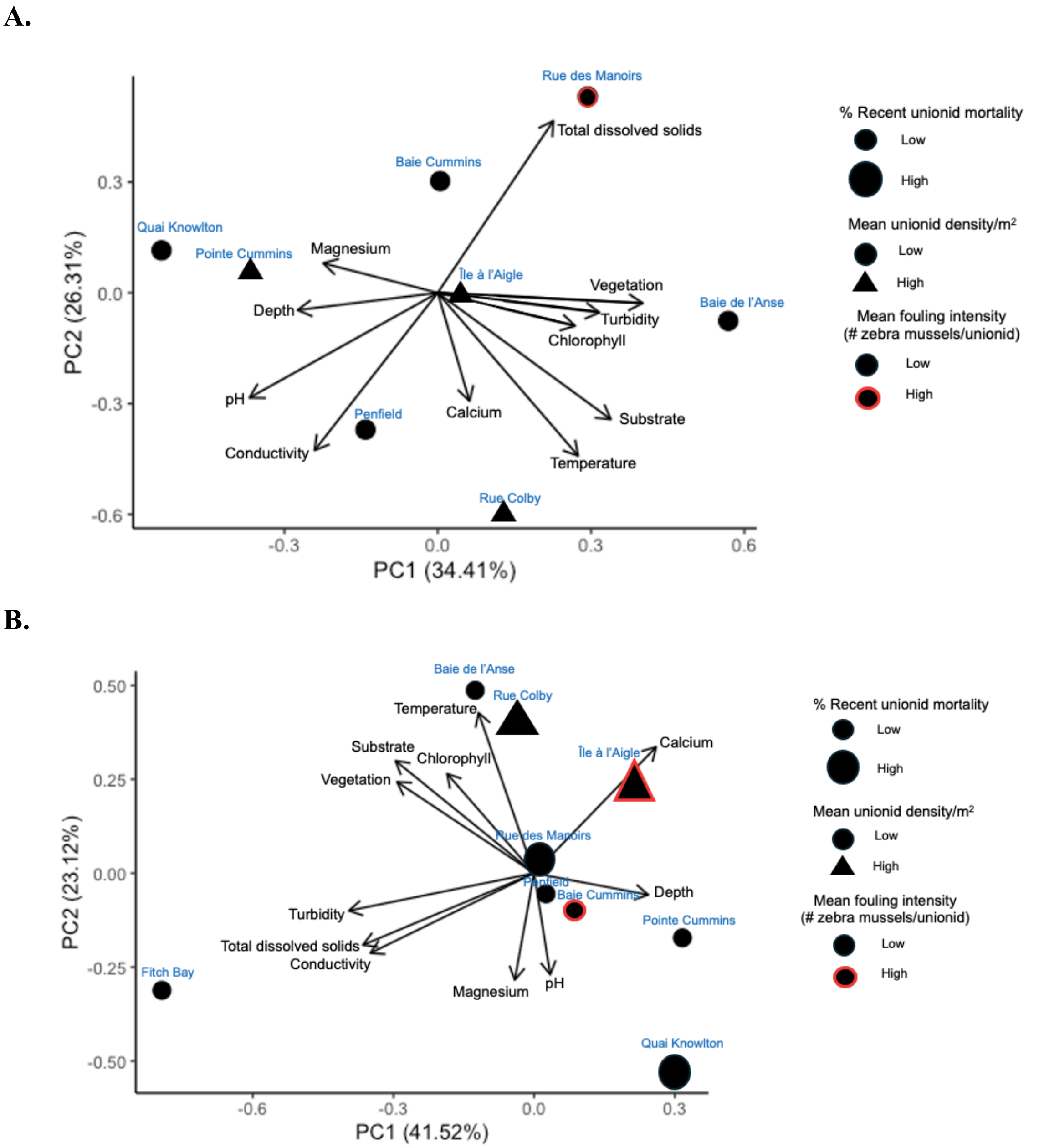


Figure S2.10. A Principal Component Analysis demonstrating summer sites in Lake Memphrémagog defined by % recent unionid mortality (low ≤ 10, high > 10), mean unionid density/m^2 (low ≤ 10, high > 10) and mean fouling intensity (low ≤ 20, high > 20) based on physicochemical variables in **A**) 2023: Vegetation (% submerged aquatic vegetation), pH, substrate (mean particle size; phi) and turbidity (NTU) had the highest contribution to PC1, whereas total dissolved solids (TDS; g/L), temperature (°C), conductivity (k cm), substrate and calcium concentration (mg/L) had the highest contribution to PC2. **B**) 2024: Turbidity, TDS, conductivity, substrate, and vegetation had the highest contribution to PC1, whereas temperature, calcium concentration, substrate, magnesium concentration (mg/L) and pH had the highest contribution to PC2.

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FINAL CONCLUSIONS

Summary of findings

Zebra mussel biofouling has caused severe declines—even extirpations—of populations of native mussels in lakes and rivers within 3 to 6 years after zebra mussel invasion (e.g., Nalepa, 1994; Ricciardi *et al*., 1996, 1998; Schloesser *et al.,* 2006). Various environmental factors are correlated with zebra mussel establishment, abundance and impact (Hincks & Mackie, 1997; Jones & Ricciardi, 2005), and some of these are relevant to impacts on unionids (Chapter 1). In most cases, mortality of unionids attributable to zebra mussels has occurred in waterbodies with calcium concentrations >25 mg/L (e.g., Gillis & Mackie, 1994; Ricciardi *et al*., 1998; Martel *et al*., 2001), which are optimal for zebra mussel reproduction and the proliferation of dense populations (Therriault *et al*., 2013) and heavy fouling (Jokela & Ricciardi, 2008). Calcium concentration can therefore be used as the key variable to predict zebra mussel impacts on native biodiversity (Jokela & Ricciardi, 2008).

By investigating the relationship between zebra mussel biofouling and unionid mortality in Lake Memphrémagog and connected downstream waters (Chapter 2), I showed that zebra mussels are capable of exerting significant impacts on native mussels in a system with what is considered to be a suboptimal average calcium concentration (~20 mg/L) for zebra mussel proliferation. Although some studies have demonstrated biofouling impacts in systems with similarly low calcium concentrations (e.g., Mellina & Rasmussen, 1994; Jokela & Ricciardi, 2005; Cusson & De Lafontaine, 1997; De Lafontaine & Cusson, 1997; Eliopoulous & Stangel, 2000), these systems are thought to be subsidized by a headwater source of larval supply where zebra mussels are reproducing in more alkaline waters. In contrast, reproduction in Lake Memphrémagog is apparently occurring *in situ* within a closed system, supplying larvae through its northerly current to downstream waterbodies (D'Avignon *et al*., 2020). Within the lake, the calcium concentration is heterogenous (ranges 16-26 mg/L); however, local calcium concentration is not correlated with biofouling in this system. Consequently, relying on risk assessments that predict establishment and impacts based on an average calcium concentration for an entire waterbody fails to consider habitat heterogeneity and context-dependencies such as the role of hydrodynamics in this system.

Significance of findings for risk assessment

My findings highlight the challenges for risk assessments that are based on environmental variables to predict dispersal ability, reproductive capacity, abundance—and, consequently, impacts of the species. In published risk assessments, Lake Memphrémagog is categorized as a "medium" risk of zebra mussel establishment where adult survival and reproduction are minimally supported (Goulet & Normand, 2023). However, I found evidence that dense adult zebra mussel populations are supported, at least locally, and co-occurring native mussel populations are suffering consequences similar to those in calcium-rich systems. Therefore, we should not assume zebra mussel impacts will be negligible at suboptimal calcium levels; instead, the effects may accrue more slowly. Although predictive risk assessments based on environmental variables can be powerful tools for anticipating the potential impact of invasive species, these assessments are most effective when used in conjunction with monitoring to avoid creating a false sense of certainty. The context dependencies of invasive species impacts require that the predictive power of risk assessments be improved through a combination of experiments and focused field surveys in presumed suboptimal and heterogeneous systems at various times since invasion.

Future directions

There is a need to monitor heterogeneous habitats, particularly those whose mean water chemistries are deemed by existing models to be low risk of severe zebra mussel impacts. Relying solely on current predictive risk assessments to anticipate invasive species establishment and impacts could results in overlooking vulnerable populations within systems, including those in proximity to other invaded lakes and rivers where propagule pressure is higher. For example, the neighboring Lake Massawippi (Québec), which has a calcium concentration in the optimal range for zebra mussel establishment, was invaded by the zebra mussel at approximately the same time as Lake Memphrémagog (i.e., 2017). Active management efforts and mitigation strategies were applied almost immediately in Lake Massawippi, whereas the same attention was not given to the presumed suboptimally low-calcium Lake Memphrémagog, which has now consequently experienced the onset of population impacts that could lead to biodiversity loss. If the benefit of predictive risk assessments is to allow for preventative measures to be implemented before the invader establishes themselves and potentially causes ecological harm, then Lake Memphrémagog is a failed case that should serve as a warning to not disregard presumed suboptimal environments.

Although there is a large body of literature to support the relationship between the calcium concentration and zebra mussel establishment, most reported biofouling cases that decimated native unionid populations were from the same decade that the zebra mussel invaded North America (e.g., Haag *et al*., 1993; Schloessor & Nalepa, 1994; Hallac & Marsden, 2000). The idea that zebra mussels are capable of exerting impacts in habitat conditions beyond their documented thresholds could become increasingly plausible in an era of rapid environmental change. Climate change may boost invasion potential, as the same traits and life-history characteristics that make a species successful in a novel environment may also allow them to respond favorably to changing environmental conditions (Dukes & Mooney, 1999; Jeffrey *et al*., 2012; Sorte, 2014). There is therefore a need to test zebra mussel threshold tolerances to habitat conditions, and to monitoring water chemistry and other environmental factors in waterbodies where zebra mussels have recently invaded.

Despite what seems like imminent peril for native mussels once zebra mussels have established in a lake or river, monitoring unionid populations in invaded systems is necessary for identifying potential sites of refugia. Natural refugia can be found in habitats where zebra mussel colonization is limited or where co-existence with zebra mussels is possible due to physical habitat characteristics (e.g., Zanatta *et al*., 2002; Bowers & Szalay, 2004; McGoldrick *et al*., 2009). In large heterogenous systems in particular, extensive monitoring of unionid and zebra mussel populations at sites of contrasting physical conditions could reveal habitats resistant to zebra mussel colonization or population growth.

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