Ecological thresholds of the Chinese mystery snail *(Cipangopaludina chinensis)* in relation to Nova Scotia environments

By
Meghan Fraser

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Approved:	Dr. Linda Campbell Supervisor
Approved:	Dr. Laura Weir Examiner
Approved:	Sarah Kingsbury Examiner
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Abstract

The Chinese mystery snail, *Cipangopaludina chinensis*, is a non-indigenous, potentially invasive aquatic species found throughout North America. They are highly fecund and viviparous, birthing live young. These features make them a potential threat to biodiversity and ecosystem function. To better understand the potential spread of *C. chinensis* in Nova Scotia, the ecological thresholds for different environmental parameters need to be established. Here, I focused on salinity, pH, and temperature using field and laboratory approaches. The salinity tolerance [(0, 5, 10, 15, and 20) ppt], and pH tolerance (pH 4, 5, 6, 7) were tested in a 2-week and 4-week laboratory experiment, respectively. The migration patterns of *C. chinensis* were monitored bi-weekly at three Halifax Regional Municipality lakes with temperature measurements. Migration monitoring was done in spring and summer, conducting surveys of the relative frequency of snails found at various depths to estimate the seasonal migration pattern of the snails. *C. chinensis* did not survive in salinity concentrations 10 ppt or higher, but did survive in 0 ppt and 5 ppt. This suggests *C. chinensis* could inhabit freshwater to brackish water. There was some mortality with juvenile *C. chinensis* in low pH conditions, and further research is recommended to establish the pH threshold for at least one life cycle of the snails. The snail migration surveys show the snails are typically found in shallow water when the surface water temperature is 20ºC and above, and in deeper water when the surface water temperature is 20ºC and below. This research suggests the ecological tolerances of *C. chinensis* may allow for the species to spread into vulnerable ecosystems in Nova Scotia not previously considered as a suitable habitat, including brackish estuaries and acidic dystrophic lakes.

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Introduction

Aquatic invasive species present a threat to biodiversity, ecosystem function, and ecosystem dynamics (Collas et al., 2017; Leuven et al., 2017). The Chinese mystery snail, *Cipangopaludina chinensis*, is a potentially invasive non-native freshwater species present throughout the United States and Canada, including the Atlantic Canadian provinces of Nova Scotia, New Brunswick, Prince Edward Island, and Newfoundland (McAlpine et al., 2016). Managing issues related to invasive species are challenging due to lack of early detection capacity, management costs, and overlapping governmental jurisdictions (Collas et al., 2017; Office of the Auditor General of Canada, 2019). A lack of public awareness in Canada limits the effectiveness of governmental management. In addition, a great majority of invasive species are spread directly and indirectly by humans and/or human activities (Burnett et al., 2018). Once established in an ecosystem, eradication of non-native species is unlikely (Collas et al., 2017). Preventing an invasive species from entering is therefore is the most efficient and cost-effective form of invasive species management (Leuven et al., 2017). Understanding the drivers that contribute to successful establishment and the potential impact a species may contribute to an ecosystem is essential for the assessment, monitoring, and management of non-native species (Leuven et al., 2017; Underwood et al., 2019). Some of the best predictors for a species' potential geographic spread into new ecosystems are their ecological tolerances, of which salinity and pH are key limiting factors in aquatic environments (Underwood et al., 2019; Ramakrishnan, 2007).

Invasive Species

Non-native, alien, exotic, introduced or non-indigenous species are synonymous terms given to any species living outside of their native habitat due to human influence (Lodge et al., 2006). The term invasive species refers to non-native species that can or have spread widely beyond their initial location of introduction and cause harm to the environment, economy or human health (Lodge et al., 2006). Invasive species can change the ecosystem function and/or ecosystem dynamics to negatively impact native species (Hintz et al., 2019). For a non-native species to develop in a new environment and become invasive, those must adapt to unfamiliar conditions and unfamiliar predators and/or prey (Colautti et al., 2006; Harried et al., 2015).

Risk assessments for invasive species typically consider four main stages of invasion: entry, establishment, spread, and impacts (Collas et al., 2017). All four stages are important to understand and research (Collas et al., 2017). The first stage, entry, has been occurring in Canada for decades (Aquatic Invasive Species Task Group, 2004; Collas et al., 2017). Today's rapid rate of non-native species introduction is cause for concern and active prevention (Aquatic Invasive Species Task Group, 2004). The second stage, establishment, is characterized by the survival of the non-native species in the new environment after they enter, before they begin to reproduce and spread (Crooks & Rilov, 2009; Collas et al., 2017). Oftentimes, native predators in the newly entered environment are not adapted to preying on non-native species, which may provide a survival advantage to the non-native species as they establish (Aquatic Invasive Species Task Group, 2004). The third stage, spread, occurs after the adults have established and have began to reproduce (Stephen et al., 2013; Collas et al., 2017). Once adapted to the environmental conditions, the juveniles of the species may begin to expand the population farther (Collas et al., 2017). The final stage of invasion, impacts, is where we see the resulting impact on the ecosystem dynamics. This is an important stage to consider when researching or managing a non-native species and will have an impact on the chosen management methods (Collas et al., 2017).

The severity of the impact on the ecosystem is directly correlated to the level of interaction between the invasive species and the native species assemblages (Harried et al., 2015). The presence of invasive species affects the persistence of native species by competing for space and/or resources, therefore limiting native species populations (Leuven et al., 2017; Underwood et al., 2019). In addition to resource competition, species such as *C. chinensis* have the ability to alter water chemistry, thus impacting native species' preferred habitat condition (Chaine et al., 2012; van Riper et al., 2019). When examining risk to the environment, some research considerations include impacts to native species and biodiversity, changes to water chemistry (especially for aquatic environments), and subsequent changes in ecosystem function (Chaine et al., 2012; Leuven et al., 2017).

When aquatic invasive species change ecosystem dynamics, they often lead to ecosystem service impacts on both social and economic scales (Escobar et al., 2018). In North America, non-native and invasive species management cost billions of dollars annually (Lodge et al., 2006; Collas et al., 2017). Disruption of food web dynamics has an effect on food chain structures; changes to species dynamics in lower trophic levels has a direct impact on larger species in higher trophic levels and vice versa (van Riper et al., 2019; Coleman, 1968). Decreases in popular sport fishing or commercial species populations can have a direct economic response, thus impacting employment and revenue on national and local scales (van Riper et al., 2019).

The Chinese Mystery Snail

The Chinese mystery snail, *Cipangopaludina chinensis*, a viviparous operculate snail, is a potentially invasive freshwater species (Harried et al., 2015). The species is edible, popular with aquarium enthusiasts, and easy to miss if attached to boating equipment; these are some of the attributed causes of wide-spread invasion by *C. chinensis* (Collas et al., 2017). In Nova Scotia, *C. chinensis* is currently not listed by the Department of Fisheries and Oceans (DFO) as an invasive species due to uncertainty about its ecological impacts, which have yet to be thoroughly studied (Chaine et al., 2012; DFO, 2015). Originally introduced to continental North America as a food item in San Francisco in the 1890s, *C. chinensis* has since spread throughout the United States and Canada (Jokinen, 1982). Now present in the Atlantic provinces (McAlpine et al., 2016), the focus of this study is based on the presence of *C. chinensis* in Nova Scotia.

Biological Features

The Chinese mystery snail may be known by several taxonomic names: *Cipangopaludina chinensis* (Grey, 1834), *Bellamya chinensis* (Grey, 1834), *Viviparus malleatus* (Reeve, 1863), or *Viviparus chinensis* (Grey, 1834). *Cipangopaludina chinensis* is popular with aquarium enthusiasts and hobbyists due to its large size and ability to feed on algae and maintain water clarity (Van der Neucker et al., 2017; Matthews et al., 2017).

Cipangopaludina chinensis has an operculum covering ("trap door") that allows the snail to seal itself in its shell during periods of desiccation, poor water quality, or duress (Unstad et al., 2013; Olden et al., 2013). During an experiment by Havel (2011), juvenile *C. chinensis* were removed from aquatic environments and exposed to dry air conditions for a period of up to four weeks. After rehydrating the snails, they observed a survival rate of 75% after four weeks of exposure. Unstad et al. (2013) conducted a drawdown experiment with adult *C. chinensis* to determine their survival while reducing the water level in the freshwater habitat. The adult *C. chinensis* survived for up to nine weeks without any exposure to food or water. There appears to be a correlation between snail size and tolerance to desiccation, with a reduced rate of survival for smaller juvenile snails (Havel, 2011). The ability to survive without access to water increases the likelihood of survival success after an aquarium release or aquarium disposal (McAlpine et al., 2016; Collas et al., 2017).

Introduction and Dispersal

The aquarium trade is believed to be one of the primary introduction pathways for the Chinese mystery snails, with aquarium release/disposal believed to increase secondary spread into new ecosystems (Collas et al., 2017; Harried et al., 2015). Dispersal of *C. chinensis* is frequently associated with boat launches (McAlpine et al., 2016). Boat launches likely make for a convenient location to dispose of aquarium snails (McAlpine et al., 2016). The species' ability to survive long periods of air exposure supports the potential introduction pathway into new habitats *via* boats and equipment moving between water bodies (McAlpine et al., 2016). Snails may get caught in or attach themselves to boats, bait-buckets, live wells, fishing gear, etc. (Matthews et al., 2017; Waltz, 2008). Chinese mystery snails, especially small juvenile snails, may be entrained in boats alongside aquatic plants and transported between water bodies (McAlpine et al., 2016). Draining, drying, and cleaning boats and boating equipment is recommended to prevent further spread by boats and related activity between water bodies (McAlpine et al., 2016; Waltz, 2008).

Ecological Impacts

Studies of the ecological impacts of *C. chinensis* in low densities within North American lakes appear to have limited impacts (Olden et al., 2013). However, the ecological impacts in high snail densities are relatively unknown (Johnson et al., 2009; Olden et al., 2013). In a mesocosm experiment (a large outdoor experiment to mimic the natural environment under controlled conditions), conducted by Johnson et al. (2009), Chinese mystery snails and rusty crayfish (another invasive freshwater species in the mid-western USA states) were confined with native freshwater snail species to assess the resulting impacts on native species when exposed to invasive species. When only *C. chinensis* were confined with native snail species, the native snails experienced a decrease in abundance and/or wet mass. When Chinese mystery snails and rusty crayfish were confined with the native snail species, the native snails experienced a decrease in both abundance and wet mass (Johnson et al., 2009). Impacts from high density populations are of particular interest, because Chinese mystery snails can occur in high densities up to 38 individuals per m^2 (Chaine et al., 2012). Because the body of evidence for the ecological impacts are still sparse, *C. chinensis* may be regarded by decision makers as either benign or detrimental, which makes prevention and management decisions difficult (Van den Neucker et al., 2017).

Chinese mystery snails can filter water as a feeding mechanism, and as such are a potentially important factor on cycling and ratios of nitrogen and phosphorus (N:P) which control algal growth in temperate freshwater lakes (Johnson et al., 2009; Olden et al., 2013). Olden et al. (2013) conducted a *C. chinensis* mesocosm experiment and found that chlorophyll-*a* (which is correlated with algae concentrations) decreased with the presence of *C. chinensis* 54% decrease in low-density treatments and a -155% decrease in high-density treatments (Olden et al., 2013). Chlorophyll-*a* did, however, increase within the substrate in the treatments containing snails in high density (40% increase in high-density treatments, 64% decrease in low-density treatments) (Olden et al., 2013).

Olden et al. (2013) also calculated an average filtration of 106-113 mL snail^{-1} h^{-1}. They also determined that mean filtration rates were more than twice as high in the high-density treatment, suggesting that individual snails filter faster when confined in a high-density environment (Olden et al., 2013). The filtration rates increased with snail size, with the exception of smaller snails less than 43.5 mm which demonstrated very little filtration (Olden et al., 2013). These filtration rates are comparable to invasive freshwater bivalves notorious for high filtering rates and subsequent freshwater ecosystem disruptions, including zebra mussels *(Dreissena polymorpha)*, quagga mussels (*Dreissena bugensis)*, Asian clam (*Corbicula fluminea)*, golden mussels *(Limnoperna fortunei)*, and blue mussels *(Mytilus edulis)* (Olden et al., 2013).

Lifecycle

Chinese mystery snails are a viviparous species, meaning that young offspring are born fully formed (Harried et al., 2015). Females can live up to five years, and males can live up to three or four years (Stanczykowska et al., 1971). Males can be identified by one short, curved tentacle that functions as a penis (Collas et al., 2017). The fecundity estimates for a single gravid female are estimated at 27-33 young per brooding season, however other studies have estimated this number as high as 100 young per brooding season (Stephen et al., 2013; Jokien 1982). A single adult female can carry a high number of embryos and is dependant on the size of the female, therefore larger snails are expected to birth more young (Jokinen, 1982). Juveniles are born as fully independent live young, typically between June and October in the northern hemisphere (Jokinen, 1982). Adults internally fertilize the embryos, and females later birth live young (though the exact brooding period is currently unknown) (Stephen et al., 2013). A female is thought to take one full year to reach maturity, after which she may reproduce for up to four years (until death) (Stephen et al., 2013). Because of their high fecundity, one single gravid female can establish an entire population in a new habitat (Van den Neucker et al., 2017). The high fecundity of the Chinese mystery snail also impacts native species by introducing a high volume of new competitors for space and resources (Chaine et al., 2012).

Feeding Mechanisms

Chinese mystery snails are facultative detritivores, they can both filter feed and graze on epiphytic diatoms, periphyton, and detritus (Collas et al., 2017). This is different from exclusive filter-feeders (i.e. bivalves) or exclusive grazers (i.e. native snail species) (Olden et al., 2013). The ability to switch between filter-feeding and grazing occurs when individuals mature; juveniles are restricted exclusively to grazing until they reach a large-enough size to filter-feed (Jokinen, 1982; Olden et al., 2013). The choice between the two feeding methods is dependant on the resources available to adult *C. chinensis* in a given environment, and the ability to switch between food-sources makes the species more competitive to not only other grazing snail species, but also to other filter-feeding mollusc species (Van den Neucker et al., 2017). It is suggested that the Chinese mystery snail may have similar impacts as invasive bivalves due to their filter-feeding, which may potentially shift microbial communities when present in high densities (Olden et al., 2013; Van den Neucker et al., 2017). Individual species have the ability to impact nutrient cycling and storage directly (via grazing and excretion) or indirectly (via predation) (Hall et al., 2003). Chinese mystery snails appear to influence the nitrogen and phosphorus ratios in impacted water bodies, modifying the ratios differently than native snails (Chaine et al., 2012). As a result, algal biomass and nutrient cycling in impacted ecosystems will change water chemistry to better suit *C. chinensis* over native aquatic species (Van den Neucker et al., 2017; Chaine et al., 2012).

Management of Invasive Species

In Canada, aquatic invasive species are managed by various departments within the government. From the Office of the Auditor General (2019) Aquatic Invasive Species: Management Action Plan:

> “While Fisheries and Oceans Canada is the lead for managing aquatic invasive species in Canada, it is a shared responsibility across numerous federal departments, including but not limited to Environment and Climate Change Canada, Parks Canada, Transport Canada, Health Canada’s Pest Management

> Regulatory Agency, National Defence, and the Canadian Food Inspection Agency."

The consulting federal department is dependant on the type of species, method of introduction, and/or practical use of the species for commercial, industrial, or residential use. The government has jurisdiction to enforce the prohibition of import, release, transport, possession, and introduction of aquatic invasive species (DFO, 2019). Once established, the government may take action to treat or destroy the invasive organisms, or temporarily prohibit access to impacted areas (DFO, 2019). In addition to the federal responsibility, aquatic invasive species are monitored by Nova Scotia Fisheries and Aquaculture on the provincial level (NS Fisheries and Aquaculture, n.d.).

Though the Department of Fisheries and Oceans (DFO) holds majority responsibility for aquatic invasive species, a recent audit from the Commissioner of the Environment and Sustainable Development to the Parliament of Canada showed DFO is "failing" to control the invasive species in aquatic habitats (Toth, 2019). The critique comes from after an audit suggesting DFO has not done their duty to prevent aquatic invasive species from entering Canadian waters, and there is no mandate to combat aquatic invasive species in the Arctic (Toth, 2019).

Management of a potentially invasive species begins with a risk assessment, considering the four main stages of invasion: entry, establishment, spread, and environmental impacts (Collas et al., 2017). Information is required at all four stages to assess for risk and subsequent impacts on the ecosystem (Collas et al., 2017). Preliminary management techniques should focus on understanding drivers to introduction and drivers to invasion success (Leuven et al., 2017). Early detection of a species is often mentioned as it is essential to prevent the entry and establishment of invasive species (Leuven et al., 2017). A major issue is that early detection of aquatic species is difficult, and they are typically only detected once populations have begun to spread (Solomon et al., 2010).

Management of aquatic invasive species is focused on preventing initial entry into an ecosystem, because eradication is often extremely challenging or impossible (Collas et al., 2017). This is confirmed by the Canadian Council of Fisheries and Aquaculture Ministers Aquatic Invasive Species Task Group (2004), who identifies managing introductory pathways to entry and spread of invasive species as the most effective approach in Canada.

Management of *Cipangopaludina chinensis*

C. chinensis has been deemed as a "high risk" species for introduction and establishment, making detection and prevention especially crucial for a number of reasons (Collas et al., 2017). The operculum feature in *Cipangopaludina chinensis* allows the snail to seal themselves within their shell and prevent exposure to harmful contaminants (Olden et al., 2013). This is a feature that protects the snail from some unfavorable environmental conditions. In an experiment by Haak et al. (2014), *C. chinensis* was not effectively killed by popular chemicals like rotenone or copper sulfate. Furthermore, eradication efforts via poison would likely unnecessarily kill valuable native organisms including fish, amphibians, and other invertebrates. Drawdowns of reservoirs and some lakes to kill undesirable species via desiccation are another management technique used to eradicate unwanted species in an aquatic environment (Burnett et al., 2018). *C. chinensis* has a high tolerance to air exposure due to their operculum feature, lasting up to nine weeks without water (Unstad et al., 2013). This management technique is unlikely to be successful in ecosystems impacted by *C. chinensis* (Burnett et al., 2018).

Other techniques may involve hand-picking individual snails out of the water (Green, 2019). For example, a New Hampshire community has resorted to custom-modified rakes to remove snails from their lake (Green, 2019). Local community members volunteer every year to rake thousands of *C. chinensis* out of their lake, motivated by the undesirable smell the snails produce (Green, 2019). This is likely an impossible task, as they occur into depths that may not be accessible without a breathing apparatus, such as SCUBA equipment (Burnett et al., 2018). Juvenile Chinese mystery snails are also relatively small and hide between rocks or burrow in sediment, making them difficult to find and remove. Failure to remove juvenile snails allows the population to rebound and potentially nullify eradication efforts. (Collas et al., 2017; Unstad et al., 2013).

Education

Educating the general public about invasive species is essential to reduce their introduction into new habitats (Leuven et al., 2017). Because some of the main introductory methods are attributed to food and aquarium industries, the pet and aquarium industry and the general public are the target audiences to prevent the intentional release of invasive species (Van den Neucker et al., 2017; Haak, 2015; Matthews et al., 2017). Boat launches are believed to be associated with *C. chinensis* because of boater traffic and the convenience for aquarium releases (McAlpine et al., 2016). As of this writing, no documents have been found guiding North American pet and aquarium trade to avoid potentially invasive freshwater species. However, the Ornamental Aquatic Trade Association (OATA) Inc. of the United Kingdom recently issued a set of recommendations for UK vendors to source only 5 locally available snail species and to avoid a number of snail species, including *C. chinensis* (OATA, 2020).

The need for specialized boater education is required to limit secondary spread (McAlpine et al., 2016). Cleaning, draining, and drying boats between uses is a recommended technique to reduce invasion from aquatic invasive species, not limited to just *C. chinensis* (Burnett et al., 2018). Current practices suggest high-pressure, 60^{o}C water used for a minimum of 10 seconds as the most efficient way to decontaminate boats from aquatic invasive species (Burnett et al., 2018). Using chemical poisons to decontaminate boats or related equipment of *C. chinensis* is not believed to be effective (Haak et al., 2014; Burnett et al., 2018). Until further research regarding the Chinese mystery snails and short-term exposure to high temperatures is available, the clean-drain-dry technique is likely to continue as the recommended method to prevent further spread (Burnett et al., 2018).

Ecological Thresholds

Knowing the ecological thresholds of an invasive species provides a narrower range of potential ecosystems, allowing for better predictions of spread into new habitats (Underwood et al., 2019). The two most obvious barriers for *C. chinensis* in Nova Scotia are salinity and pH. Salinity is a key factor in determining the spread of aquatic species, as it affects physiological functions (Underwood et al., 2019). The ability of a freshwater species to survive in saline conditions will determine their potential to enter and establish in estuarine or brackish environments (Ramakrishnan, 2007). In Nova Scotia, most freshwater lakes connect with the oceans, so significant salinity gradients can be found around the province in estuaries and other mixed-water sites. Salinity could be an important barrier for *C. chinensis* movement into salt-water coastal areas and between lakes connecting through estuaries.

The other key ecological threshold is pH. Aquatic biodiversity decreases with decreasing (acidic) pH; acidic environments are not usually preferable for inhabitation (Ramakrishnan, 2007). For species with calcified shells or exoskeleton, an acidic environment limits the available calcium carbonate ($CaCO_3$) needed to form their shells (Latzka et al., 2014). There are more than 3000 lakes in Nova Scotia, with hundreds of these lakes fall into a pH range between 4.0 and 7.0 (Nova Scotia Lake Inventory Program; Coleman, 1968). Narrowing the preferred range of *C. chinensis* in waters with varying pH will drastically decrease the potential habitats available for the snails to enter and establish (Latzka et al., 2015) and aid with identifying potential priority sites for *C. chinensis* monitoring (Kingsbury et al., in prep.).

Generalist species, such as Chinese mystery snails, have the ability to adapt to new environmental conditions which increases their invasion potential (Burnett et al., 2018). Even so, Chinese mystery snails are native to eastern Asian habitats, and the environmental conditions present in Nova Scotia are likely different from those in their native range, including temperature, salinity, and pH regimes (Jokenin et al.,1982; Burnett et al., 2018). Thermal tolerance is a well-established constraint to the geographic range a species can occupy (Burnett et al., 2018). The temperatures in North American dimictic lakes generally increase in the spring and summer months and then decrease in the fall and winter months following the typical biannual stratification pattern. *C.*

chinensis in dimictic lakes of North America have been observed to move into deeper water during the cool fall months in order to escape mortality from the freezing temperatures near the surface (Burnett et al., 2018; Jokinen, 1982). They begin to move closer to shore when the temperatures increase in the spring and can be found very close to the surface during peak summer temperatures (Jokinen, 1982; Burnett et al., 2018).

Methods

Snail collection & Culturing

Before the experiments were started, a *C. chinensis* culture was established in the aquatic invertebrate laboratory at Saint Mary's University. Approximately sixty adult Chinese mystery snails were collected by hand from Loon Lake (Dartmouth, NS) in June 2019. Loon Lake is a freshwater urban lake with a boat launch and a well-developed shoreline adjacent to highways and housing *C. chinensis* were first reported for Loon Lake in 2014 (McAlpine et al., 2016), and monitored annually by Dr. Linda Campbell (pers. Comm., Environmental Science, Saint Mary's University). The collected adult snails were scrubbed with a toothbrush, placed in an aquarium with dechlorinated water and sand collected from Loon Lake. Calcium was added to the dechlorinated tap water on Fridays. Water was changed and replaced with dechlorinated tap water three days per week. The snails were fed commercial aquarium algae food pellets three days per week (Monday, Wednesday, Friday).

When juvenile snails emerged, those were removed from the aquarium and relocated into glass jars as weekly cohorts that were monitored daily. The juvenile snails were kept in either 1-liter jars (10 juveniles per jar) or 2-litre jars (20 juveniles per jar).

The jars were kept in a water bath set-up with temperature controls (i.e. water heaters) to maintain consistent conditions. The jars were maintained with algae pellets, water changes, and calcium addition at the same rate as the adult aquarium. Prior to the laboratory (salinity and pH) experiments, the shells of both juvenile and adult snails were marked with one of four nail-polish colours for identification and tracking purposes, allowed to dry, and then placed back into their original culture aquarium.

Salinity Experiment

To determine the salinity threshold of the Chinese mystery snail, a salinity experiment was conducted. This experiment consisted of five treatments of varying saline concentrations [(0., 5, 10, 15, 20) ppt] for both adult and juvenile *C. chinensis*, with three replicates of each treatment.

The saline water treatments were produced in 20-L carboys by combining Instant Ocean© salt mix and 10-L dechlorinated tap water, in four separate concentrations. The control treatment, 0 parts per thousand (ppt), consisted of dechlorinated tap water without added salt mix. The 5 ppt (58.3g of salt mix), 10 ppt (116.6g of salt mix), 15 ppt (174.9g of salt mix), and 20 ppt (233.2g of salt mix) treatments were each shaken with 10-L of dechlorinated tap water in the carboys to adequately dissolve the salt mix into the water. No additional calcium was added to the carboys. Each carboy was checked with a multi-parameter YSI © probe to note the salinity, dissolved oxygen, total dissolved solids, conductivity, pH, and temperature.

The experimental system consisted of thirty 946-mL jars placed in water baths with temperature control measures (water heaters, thermometers). One large water bath contained fifteen jars, and three smaller water baths each contained five jars. Each jar was equipped with air hose connected to an air pump, mesh netting, and a clip to keep the air hose in place. Lights were set up above each water bath and controlled to a similar output between 100 and 1000 lux. The lights were kept on a timer to allow for 8 hours of darkness per day. The jars were filled with one of five water treatments. In the large water bath, replicates of each treatment were grouped in similar areas within the bath. Adult snails were housed in the large, 30-jar water bath. Juvenile snails were housed in the three smaller water baths, with jars placed so that each bath held one replicate per treatment.

The experimental system was set up on July 6, 2019 and allowed to sit until July 8, 2019 when the live snails were added to the system. The water chemistry was checked in each of the jars before the addition of the live snails. Each of the colour-coded snails were weighed and measured with a digital scale and digital caliper before being sorted into the experiment. Snails were divided into jars, allowing for some randomization between cohorts, and the nail polish colour on their shell (i.e. only one of each colour per jar). There were two adults per jar, for a total of six adults per treatment. Four juvenile snails were added per jar, for a total of twelve juveniles per treatment. The snails were not fed during the 14-day experiment.

The experimental jars were checked daily five days per week, at the same time of day. The water was changed in each jar twice per week (Monday and Thursday) for a 50% change per week, and the water chemistry data was collected on Monday during the water change with a YSI© multi parameter probe. During the daily checks, the water temperature was noted, and snails were checked for mortality. If mortality was evident, the snail was removed from the system with a sterile spatula and logged.

This experiment was terminated on July 22, 2019, after a two-week period. It was terminated because mortality was high in the high saline treatments, as indicated by floating organisms. All snails were removed from the system, weighed and measured, and placed in a plastic bag in the freezer. Water chemistry data was collected from the jars with the YSI probe upon removal.

pH Experiment

The water treatments used in this experiment were collected from lakes spanning a range of known pH in the Halifax Regional Municipality (HRM). The pH was verified with a multi-parameter YSI probe during lake water collection. These lakes included: Second Chain Lake, Little Indian Lake, Chocolate Lake, and Cow Bay River. Lake water was collected from the lakes on August 12, 2019 and August 14, 2019. The chosen collected method required a 10-litre carboy to pour the lake water over a fine-grain metal sediment sieve into a 20-litre carboy. Water was collected again from all lakes on September 6, 2019 and September 14, 2019 to replenish the diminishing in-lab supply, and the pH was verified for consistency.

In addition to these natural lakes, dechlorinated tap water and two pH buffer solutions [HANNA Instruments pH buffer solution, HI 70007 (pH 7.33) and HANNA Instruments buffer solution, HI70004 (pH 4.35)] to replicate a similar experiment reported by Haak (2015). No additional supplements, such as calcium, were added to the water for the experiment. Each natural water and tap water treatment were given three replicates for both adult and juvenile snails. For the pH buffers, due to limited quantities of buffer solution, only juvenile snails were used due to their vulnerability. Three water baths were set up with similar water heaters and thermometers. Each jar was equipped with air hose connected to an air pump, mesh netting, and a clip to keep the air hose in place. Lights were set up above the water baths and set to an output between 100 and 1000 lux. The lights were kept on a timer to allow for 8 hours of darkness per day. Fifteen 946-mL jars were placed in two of the water baths and filled to the bottom lip with either dechlorinated or lake water. Six 946-mL jars were placed in a smaller water bath and filled with the pH buffer solution mix. The jars were checked with an EcoSense© pH probe and a YSI© multi-parameter probe to record the same water chemistry data monitored in the previous salinity experiment. The single pH probe was used to ensure consistency during the experiment as it fitted within the jars more easily. The experiment was set up and allowed to sit for three days before live snails were added to the system.

On August 22, 2019 the snails were weighed and measured before being placed into one of the pH treatments. Two adult snails or four juvenile snails were placed in each jar, sorted according to nail polish colour on their shells.

The experiment was checked daily five days per week, at the same time of day. The water was changed in each jar twice per week (Monday and Thursday) for a 50% change per week, and the water chemistry data was collected on Monday during the water change with a YSI© multi parameter probe. pH data was collected on both Mondays and Thursdays with the EcoSense© pH probe to closely monitor any changes. The snails were not fed for the duration of this experiment.

During the daily checks, the water temperature was noted, and snails were checked for mortality. If mortality was evident, the snail was removed from the system with a sterile spatula. The experiment was terminated on September 19, 2019, after 28 days. All snails were removed from the system, weighed and measured, and placed in a plastic bag in the freezer. Final water chemistry data was collected from the jars with the YSI© probe.

Temperature Migration Surveys

Shoreline surveys were conducted on a bi-weekly basis from May until September. The data was collected from sites within the Halifax Regional Municipality (HRM) already known to have high densities of *C. chinensis*: Sullivan’s Pond, Lake Banook, and Loon Lake. First, a multi-parameter YSI© probe was used to collect the water chemistry data from the survey site. The parameters of interest were water temperature, salinity, dissolved oxygen, total dissolved solids, conductivity, and pH. In addition to water chemistry data, site weather and physical conditions were also noted. Then the surveys were done in pairs, with one surveyor in the water and the notetaker on shore. The surveying method was adapted from the stream sampling method established by the Canadian Aquatic Biomonitoring Network (CABIN) and random stratified sampling efforts were adapted from Strayer & Smith (2003).

The snail surveys were conducted by the snail surveyor in the water with an underwater scope (a 20-gallon white paint bucket with a transparent plastic bottom) and a lead-weighted line with marked 0.15-m intervals. The surveyor started at the assigned starting location and would walk away from the shoreline. Through the underwater scope, the surveyor would search the substrate and call out the notetaker the depth of the snail, and the number of snails. The survey was concluded when the data from ten snails was recorded, the survey exceeded the twenty-minute maximum duration, or exceeded a 100-m maximum transect distance. Three surveys were completed in each of the three lakes, and repeated bi-weekly.

Results

Salinity Experiment

Adult and juvenile *C. chinensis* were placed in 5 saline treatments of 0 ppt (control dechlorinated water), 5 ppt, 10 ppt, 15 ppt, and 20 ppt, and monitored for 14 days. The final wet mass (upon mortality or end of the 14 day-experiment) for each adult *C. chinensis* compared to their initial wet masses (n=30) show a decreasing trend with higher salinity (Figure 1; Appendix A; p-value = 0.01828). The snails in the 10-ppt, 15-ppt, and 20-ppt salinity treatments showed a significant decrease in mass between the commencement of the experiment and snail mortality (Figure 1; Appendix A; p-value = 0.00102). The snails' average final wet masses had very little change over time in the 0-ppt control and in the 5-ppt treatment despite not being fed during the duration of the experiment (Figure 1).

Adult *C. chinensis* were exposed to saline treatments for a period up to 14 days, with deceased individuals removed upon discovery. The duration in experiment, in which the data points represent the number of days *C. chinensis* were exposed to the experiment, were grouped by saline treatment (Figure 1). All *C. chinensis* in the 0-ppt control and in the 5-ppt treatment remained alive for the entire duration of the experiment (Figure 1). The 10-ppt treatment had 0% survival by day 9 (Figure 1). The 15-ppt treatment showed 0% survival by day 14 (Figure 1). The 20-ppt treatment showed 0% survival by day 4 (Figure 1).

The initial wet mass of juvenile *C. chinensis* (n=60) were recorded prior to the commencement of the 14-day salinity experiment (Figure 2). Final wet mass was recorded upon removal from the experiment, either upon mortality or at the end of the 14-

day period (Figure 2). The average final wet mass of juvenile *C. chinensis* show a trend that suggests the treatments of higher salinity are correlated to a greater decrease in mass (Appendix A; p-value = 0.0545). The 10-ppt, 15-ppt, and 20-ppt salinity treatments showed a decrease in the average final wet mass, when compared to average initial wet mass per corresponding treatment (Figure 2). The final average wet mass in the (0-ppt) control and in the 5-ppt treatment presented little change between initial wet mass per corresponding treatment (Figure 2).

Juvenile *C. chinensis* were exposed to saline treatments for a period up to 14 days, with deceased individuals removed upon discovery. All *C. chinensis* in the 0-ppt control remained alive for the entire duration of the experiment (Figure 2). The 5-ppt treatment had 83% survival by day 14 (Figure 2). The 10-ppt and 15-ppt treatments had 0% survival by day 14 (Figure 2). The 20-ppt treatment showed 0% survival by day 7 (Figure 2).

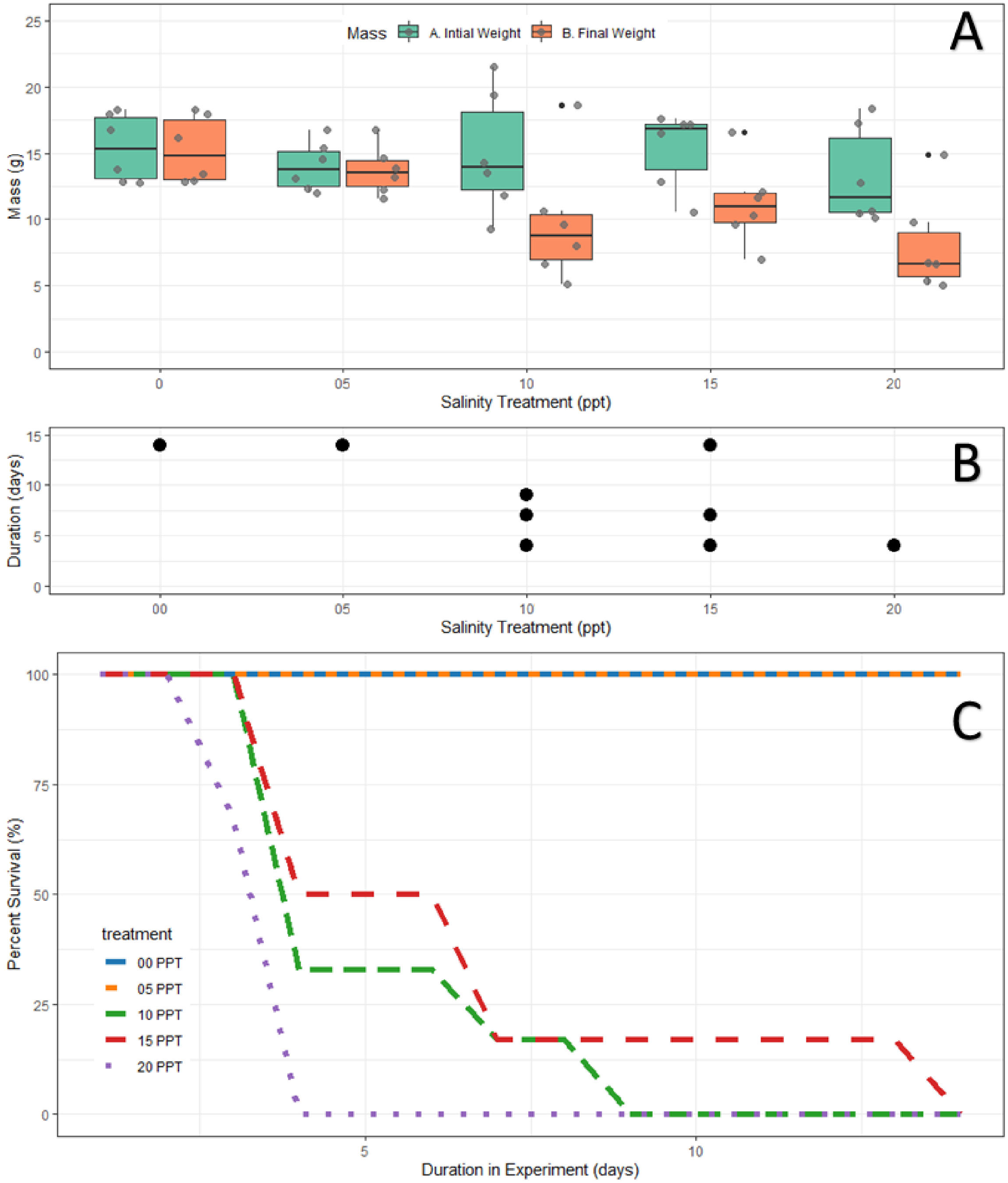

Figure 1. (A) Boxplot graph comparing the initial mass (g) to the final mass (g) of adult *C. chinensis* (n=30) in a range of salinity concentrations from 0 to 20 ppt after ≤ 14 days of exposure. (B) Duration of time (days) adult *C. chinensis* were exposed to salinity concentrations from 0 to 20 ppt until removal due to mortality (≤ 14 days) or due to the completion of the experiment (at 14 days). (C) Percent survival of adult *C. chinensis* over a 14-day exposure period to a range of salinity concentrations from 0 to 20 ppt.

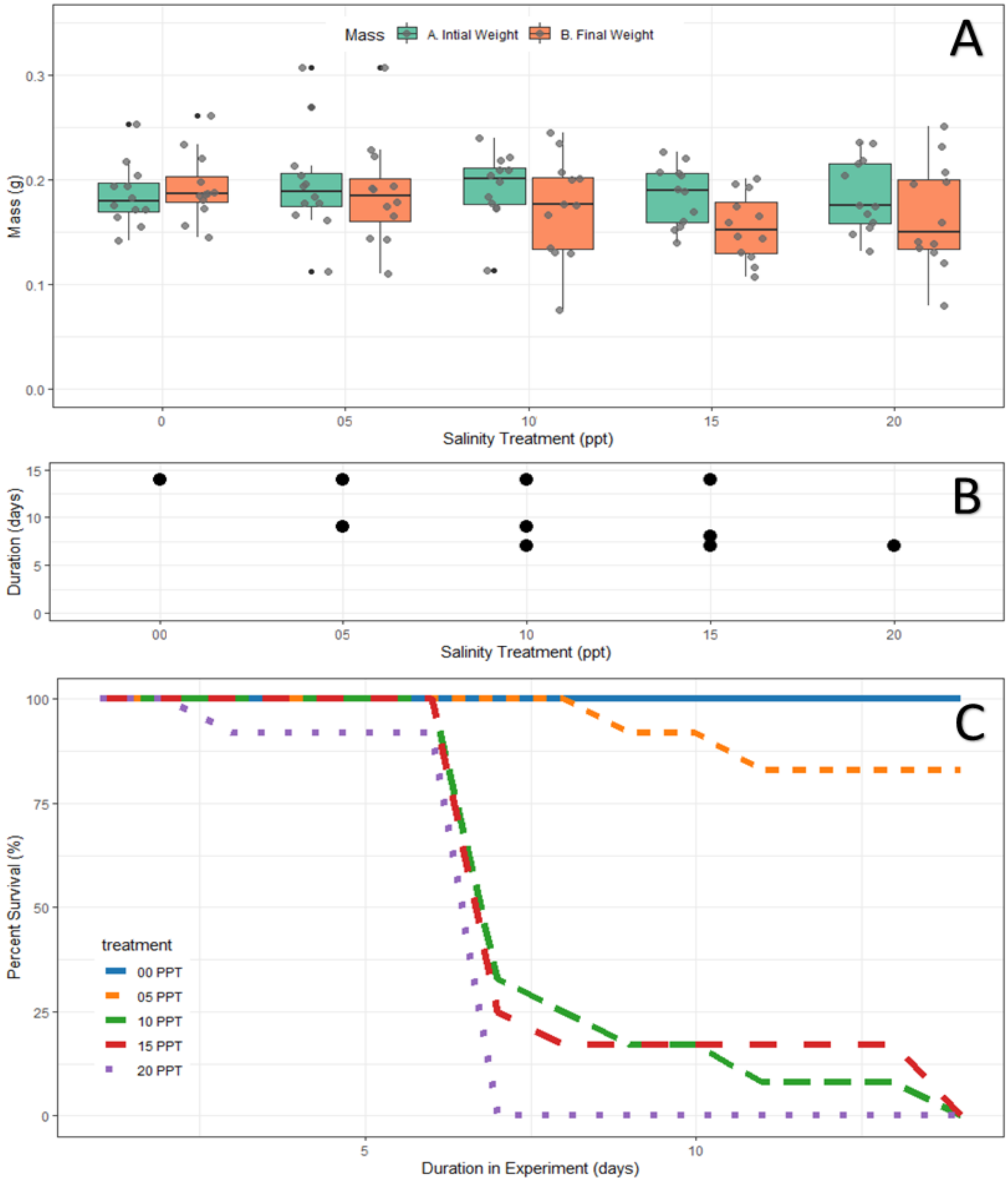

Figure 2. (A) Boxplot graph comparing the initial mass (g) to the final mass (g) of juvenile *C. chinensis* (n=60) in a range of salinity concentrations from 0 to 20 ppt after ≤ 14 days of exposure. (B) Duration of time (days) juvenile *C. chinensis* were exposed to salinity concentrations from 0 to 20 ppt until removal due to mortality (≤ 14 days) or due to the completion of the experiment (at 14 days). (C) Percent survival of juvenile *C. chinensis* over a 14-day exposure period to a range of salinity concentrations from 0 to 20 ppt.

pH Experiment

Adult and juvenile *C. chinensis* were placed in a range of water pH, sourced from various lakes in the Halifax Regional Municipality (HRM). The initial pH range for each water source was 4.35 (Second Chain Lake), 5.33 (Little Indian Lake), 5.49 (Chocolate Lake), 6.87 (Cow Bay River), and 7.81 (dechlorinated tap water), recorded prior to the start of the experiment. The pH in each of the water treatments became increasingly neutral over time, likely due to the calcium in the snail shells, snail excretions, and through exchange with the atmosphere. The majority of pH water treatments concluded with a pH between 7 and 8, with the exception of the juvenile 4.35 treatments, which concluded with a pH between 5 and 6. The initial wet mass of adult *C. chinensis* (n=30) were recorded prior to the commencement of the 28-day pH experiment (Figure 3). Final wet mass was recorded upon removal from the experiment, at the end of the 28-day period (Figure 3). There was no significant difference between the initial and final wet mass of adult Chinese mystery snails per corresponding treatment (Appendix B; p-value = 0.8749). All adult snails survived the entire 28-day period despite not being fed.

In addition to four lake pH treatments and a dechlorinated tap water treatment, juvenile *C. chinensis* were exposed to two buffer treatments (4.01 and 7.01 buffer solutions, initial pH of 4.35 and 7.33) based on a similar experimental protocol for *C. chinensis* (Haak, 2015). In this dataset, data from the two buffer treatments were combined into one data series (identified as “Buffer” in Figure 4). The datasets were combined due to similarities in the change in final wet mass and the 0% survival rate within the buffer solution treatments (Figure 4). The initial wet mass of juvenile *C. chinensis* (n=84) were recorded prior to the commencement of the 28-day pH experiment (Figure 4). Final wet mass was recorded upon removal from the experiment, either upon mortality or at the end of the 28-day period (Figure 4). There was no significant difference between initial and final average wet mass of juvenile Chinese mystery snails per corresponding treatment (Appendix B, p-value = 0.8249). Snails in the 4.35 pH treatment experienced mortality at day 4, with 25% survival by day 28 (Figure 4). Snails in the buffer treatments had 0% survival at day 14 (Figure 4). Juvenile snails in the 4.01 buffer solution treatment showed disfigured shells after 14 days of exposure to the acidic buffer solution (Figure 5). This disfigurement appeared as a deflated “bubble” shape on the surface of the shells (Figure 5), which was not apparent on the shell surface in any other pH treatment (Figure 5).

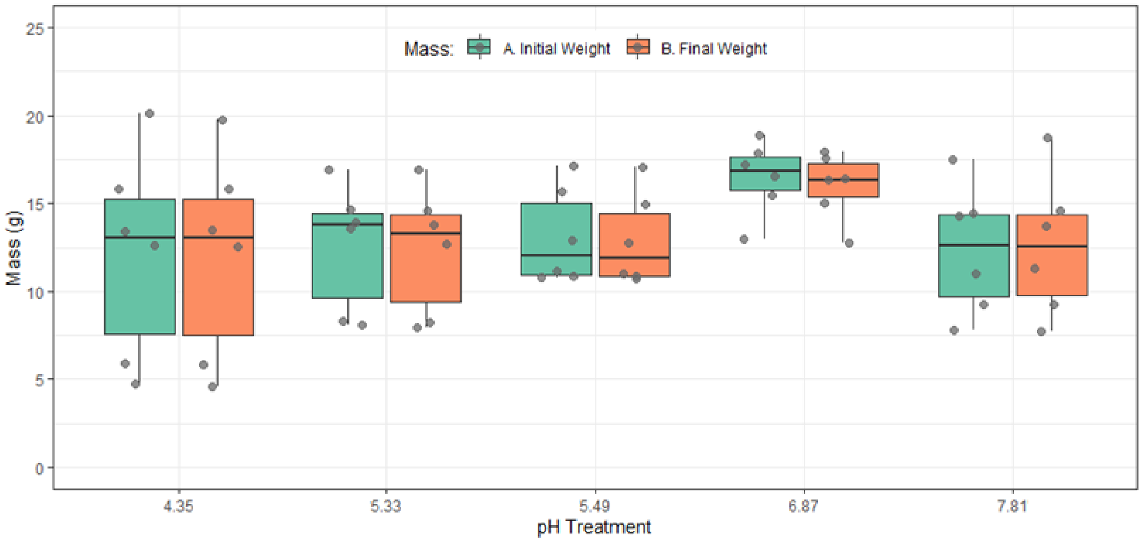

Figure 3. Boxplot comparing the initial mass (g) to the final mass (g) of adult *C. chinensis* (n=30) in pH treatments with an initial pH of 4.35, 5.33, 5.49, 6.87, and 7.81 after 28 days of exposure.

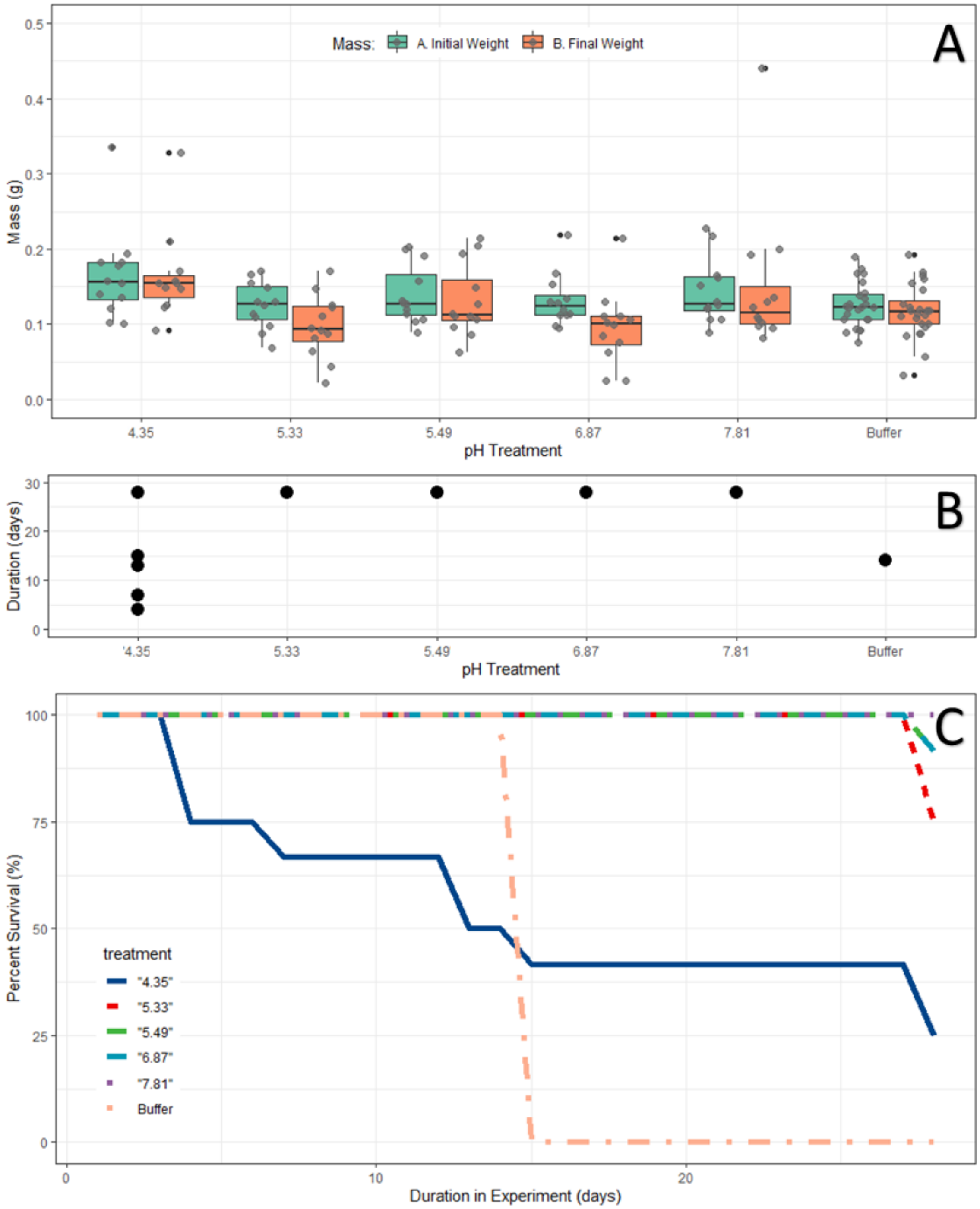

Figure 4. (A) Boxplot comparing the initial mass (g) to the final mass (g) of juvenile *C. chinensis* (n=84) in pH treatments with initial pH values from 4.35 to 7.81 after ≤ 28 days of exposure. An outlier (0.94g) for a large juvenile snail was removed from the 4.25 pH treatment 'Final Weight' in order to show the ranges of the masses for all treatments. (B) Duration of time (days) juvenile *C. chinensis* in pH treatments with initial values of 4.35 to 7.81 plus two buffer solutions, after mortality (≤ 28 days) or due to the completion of the experiment (at 28 days). (C) Percent survival of juvenile *C. chinensis* over a 28-day exposure period with initial pH values of 4.35 to 7.81 plus two buffer solutions.

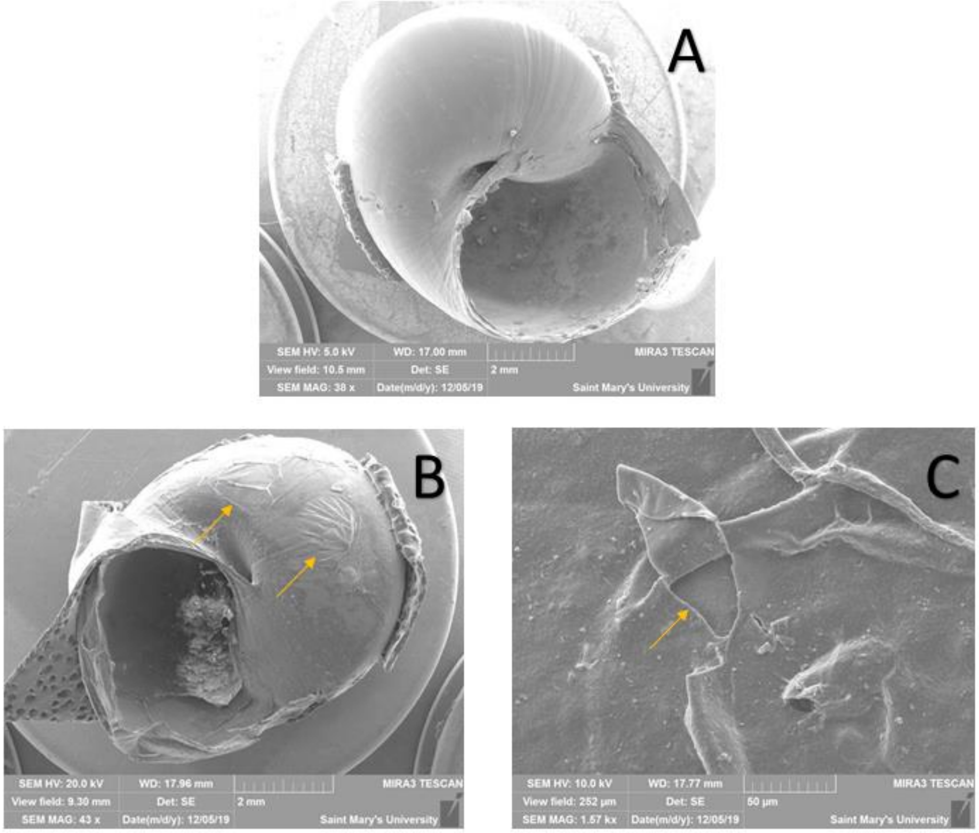

Figure 5. (A) Scanning electron microscope (SEM) image (under 38 times magnification) of a juvenile Chinese mystery snail shell with no apparent surface damage after 28-day exposure to dechlorinated tap water (initial pH 7.81). (B) SEM image (43 x) of a juvenile Chinese mystery snail shell surface after 28-day exposure to 4.01 pH buffer solution (initial pH 4.35, final pH 5.38). The yellow arrows indicate areas of visible damage to the covering periostracum layer. (C) SEM image (1,570 x) of a juvenile Chinese mystery snail shell surface after 28-day exposure to 4.01 pH buffer solution (initial pH 4.35, final pH 5.38). The yellow arrow indicates area of damage to the covering periostracum layer and the exposed top portion of the calcareous ostracum layer.

Temperature Migration Surveys

Bi-weekly lake surveys were conducted at three urban lakes known to have high densities of *C. chinensis*: Loon Lake, Lake Banook, and Sullivan's Pond. Timed migration surveys were conducted to determine the depth at which adult *C. chinensis* were found for each sampling visit. Snails were present at greater depths when water temperatures were low, and at shallower depths when water temperatures were high (Figure 6). The late spring and early fall had the coolest water temperatures, and the summer had the warmest water temperatures.

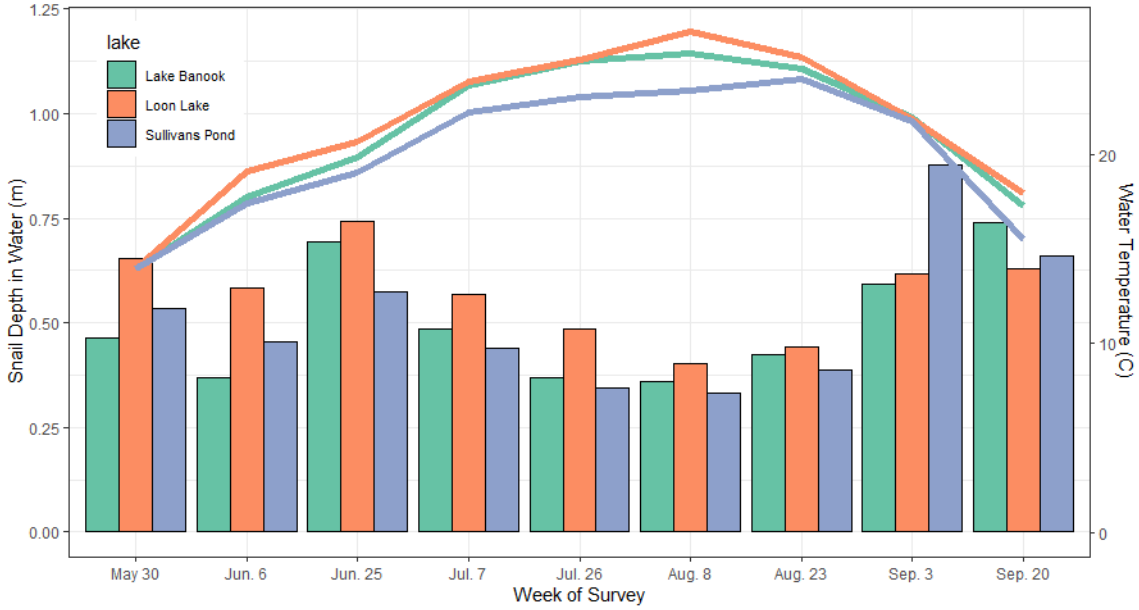

Figure 6. Bi-weekly shoreline surveys in Loon Lake, Lake Banook, and Sullivan's Pond in Dartmouth, Halifax Regional Municipality. This shows the average *C. chinensis* depth for all observed snails as per survey protocol and the surface water temperature measured at the time of the survey.

Discussion

Salinity Experiment

Nova Scotia has over 6,000 lakes, where most are connected to the ocean (Museum of Natural History staff, 1996). As a result, salinity is an important ecological threshold for many freshwater and marine species in Nova Scotia with only a few diadromous and euryhaline species able to cross this threshold. It is important to assess whether *C. chinensis* are capable of movement across salinity gradients and the risk to vulnerable coastal environments. Our experiments show that the 10-ppt, 15-ppt, and 20-ppt salinity treatments were quite toxic, with high mortality within 14-days of exposure. In addition, the final average wet mass showed a clear trend displaying a decrease in mass within the high salinity treatments (Figure 1). Mortality and loss of mass (Figure 1) are strongly suggestive that *C. chinensis* are not able to establish in high-salinity environments such as marine environments and highly saline water bodies, and the decrease in wet mass supports this (Figure 1). However, the individual data variability for snail wet masses in the 10-ppt treatment highlights the wide range in adult *C. chinensis* wet mass as they approach the ecological threshold and the potential for individuals to be able to tolerate this level of salinity. As a result, aquatic ecosystems in Nova Scotia with salinities up to 10 ppt should be included in monitoring and risk assessment programs in the future. To place this in context, there are many estuary and mixed-water tidal systems such as the Annapolis River in Nova Scotia, that have salinity gradients 0 to 25 ppt within a single system (Museum of Natural History staff, 1996) and oceans typically have salinities around 35 ppt.

Some of the variability in the final wet mass in the salinity experiments, especially at the higher concentrations, are likely due to the condition of the adult carcass upon removal from the experiment. When mortality was obvious (i.e. change in shell appearance, floating in water, cloudy water, etc.), the deceased individual was removed from the treatment. In some cases, the organisms were in advanced stages of decay in which the carcass would detach itself from the shell. Some portions of the organisms may have been lost in cases where the carcass detached from the shell before proper removal was possible. All distinguishable mortalities were removed as quickly as possible from the experiment to protect any living participants from exposure to sickness or otherwise influence their survival in the experiment.

However, snails with closed shells that were not floating can be difficult to assess for mortality, so a few snails may have been overlooked for a short time as a result. For example, the logged percent survival of the adult *C. chinensis* in the 10-ppt, 15-ppt, and 20-ppt treatments were all 0% by day 14. The snails in the 20-ppt treatment were the first to reach 0% survival, followed by the 10-ppt treatment. Only one adult remained in the 15-ppt treatment until day 14, which was confirmed as deceased upon removal. Removal from the experiment was only done when mortality was obvious, therefore the single remaining 15-ppt adult was kept in the experiment until confirmation of mortality could be established.

The adult *C. chinensis* in the 0-ppt control treatment showed very similar results to the 5-ppt salinity treatment when comparing final wet mass to initial mass of the corresponding treatment. Snails in the 5-ppt treatment also had 100% survival after 14 days of exposure. This suggests that adult *C. chinensis* may survive in brackish environments of low salinity. The potential for establishment in brackish environments is a potential threat to ecosystems that may not have been considered previously as most North American studies have focused on land-locked freshwater ecosystems to date.

Juvenile *C. chinensis* showed similar patterns to the adult *C. chinensis,* with average change in wet mass and percent survival decreasing with increasing salinity levels (Figure 4, Figure 1; Figure 6, Figure 3). Juvenile *C. chinensis* were used in addition to the adults, because the juvenile snails are thought to be more vulnerable to environmental extremes. It was clear the high salinity treatments (10-ppt, 15-ppt, and 20-ppt) were uninhabitable for all juvenile snails, with 0% survival on day 14 (Figure 6). There were obvious decreases in average final wet mass, though it was not statistically significant due to small sample numbers (Figure 4). The decrease in wet mass were similar to those in adult saline experiment, in which the highly saline treatments showed a greater change than the 0-ppt control and 5-ppt treatment (Figure 4). The snails in the 5-ppt treatment had an 83% survival on day 14 (Figure 6), with a fairly consistent average mass from initial wet mass to final wet mass (Figure 4). Though the juvenile snails in the 5-ppt treatment showed some mortality, an 83% survival rate after 14 days exposure suggests juvenile *C. chinensis* may survive and establish in low-saline brackish environments which speaks to an increased risk for population establishment in those environments.

pH Experiment

In Nova Scotia, freshwater lakes can range from less than 5 to more than 7 (Museum of Natural History staff, 1996). pH is a natural barrier for shelled molluscs, as acidic conditions limit available calcium for shell development (Weyhenmeyer et al., 2019). Adult *C. chinensis* showed little change in wet mass across all pH treatments and had a survival rate of 100% over 28 days. *C. chinensis* were placed in water sourced from different lakes in the Halifax Regional Municipality (HRM) with the initial pH ranging from 4.35 to 6.87 and dechlorinated tap water with pH of 7.81. The pH was monitored daily, and the pH became more over time. By the end of the 28 days, the pH was relatively neutral in all treatments. This suggests *C. chinensis* may be altering the water chemistry to better suit their preferred conditions. We did not use any buffering for those experiments, although we did exchange water regularly. We suspect that shell material and the snail excretion rates are affecting water chemistry, especially given the small volumes in those jars (please see below regarding the results from the buffer solution experiment).

Juvenile *C. chinensis* were exposed to the lake treatments as well as two pH buffer solution (pH 7.33 and pH 4.35). The average initial and final wet mass for surviving snails remained consistent across all treatments, including dechlorinated water, with some increase in mass resulting in a few outliers (Figure 4). Some juveniles showed an increase in mass in the 4.35 pH (Second Chain Lake) treatment, but the percent survival was 25% by day 28 (Figure 4).

As with the salinity experiment, any obvious mortalities were removed as soon as those were noticed. Some of the dead juvenile snails may have been overlooked due to their small size and closed operculums which hindered visual inspection of status in each experimental beaker. Nonetheless, trends can be discerned with the snails in the 5.33 pH (Little Indian Lake) treatment which had a percent survival of 75%. The snails had higher survival rate with 91.7% by day 28 in both the 5.49 pH and 6.87 pH (Cow Bay River) treatments (Figure 4).

The choice to use the pH buffer solutions was influenced by Haak (2015), who had used pH buffer solutions to determine the impacts on adult *C. chinensis*. Note that Haak (2015) did not use any juveniles due to rearing difficulties in a laboratory setting so results are not directly comparable. Haak (2015) reported that the pH did not have an affect on growth (measured in shell length) or wet mass, and all Chinese mystery snails survived the 4-week period. Even so, the findings in this study are different from the findings in Haak (2015), as the survival rate was 0% after 14 days (Figure 4), compared to 100% survival after their 4-week period. This difference in survival rates is likely due to a few factors: the juvenile *C. chinensis*, given their smaller sizes and thinner shell structures, are more vulnerable to harsh conditions, pH buffer solution chemistry, or differences in experimental design. Juvenile snails are more susceptible to harsh conditions as they are much smaller than adults and have weaker shells, their shells act as a protective barrier. The impact of the pH buffer solution on shell structures can be clearly visualized by SEM imaging (Figure 5), with apparent bubbling occurring under the brown-pigmented outer periostracum leading to separation of layers. This would potentially increase the vulnerability of the inner calcareous ostracum layers to acidic conditions. The experiment was not continued long enough to observe whether damage could reach the hypostracum layers. In this experiment, snails in the acidic 4.01 pH buffer (initial pH of 4.35) and the neutral 7.01 pH buffer (initial pH of 7.33) had a survival rate of 0% after 14 days, suggesting the cause of mortality was the pH buffer solution, and not due to pH. Snails in the 4.01 buffer solution treatment showed apparent shell damage upon removal from the experiment. The pH buffer solution preparations may have influenced the rate of survival and made the juvenile snails vulnerable, as the composition in the water treatments likely vary. It is highly recommended that commercial pH buffer solution is not used for this type of experiment and alternative approaches be found for future pH studies.

While there was 100% survival in all adult snail pH treatments, there was mortality in juvenile snail pH treatments. The acidic initial pH of 4.35 had a percent survival of 25% with juvenile snails, which indicates establishing populations of the species would be delayed in low pH environments through attrition of offspring. In other words, the adults may survive and live in acidic conditions and the decreased survival rate of juvenile snails delay or prevent establishment success in new environments with low pH. Because *C. chinensis* are a species with relatively large shells, the calcium stores within the shells may provide enough calcium for individual survival and fecundity in acidic waters up to a point. It is unknown of internal calcium supplies and maternal transfer of calcium to embryos can sustain multi-generational populations over time, however. Multi-generational laboratory studies may be required to further understand the pH tolerances of this species on a population level.

Temperature Migration Surveys

Bi-weekly temperature migration surveys were conducted during the summer season at three urban lakes in Dartmouth, Halifax Regional Municipality, Nova Scotia. Using and adapted timed mussel survey method, adult *C. chinensis* individuals were counted and their respective depths they were recorded. Overall, average depths for adult *C. chinensis* were deeper (> 0.5 m) when the water temperature was less than 20℃ and became shallower (< 0.5 m) when the water temperature reached 20℃ or above (Figure 6). This is in alignment with Jokinen (1982)’s observations of *C. chinensis* movements towards shorelines in the Spring and movements back into deeper water in October where snails tend to remain through the winter. However, the snails in our lake surveys indicated that those start their movement back to deeper waters earlier in September rather than October (Figure 6). The difference in movement timing is likely due to the more northern latitudes of our lakes compared to Jokinen's study lakes resulting in earlier turnover and stratification. As temperature regimes and lake stratification periods appear important to *C. chinensis* in northern temperate lakes, temperature should be considered a key parameter incorporated in future monitoring programs, along with salinity, pH, and bioavailable calcium.

Conclusions

The salinity threshold of *C. chinensis* appears to exist between 5 ppt and 10 ppt. The experiment conducted strongly suggests salinity as a natural barrier for *C. chinensis*, as neither juvenile nor adult snails survived in the high saline treatments (10 ppt, 15 ppt, and 20 ppt treatments) for a period of more than 14 days. It is possible that a brackish, low salinity (up to 5 ppt) environment may support the survival and establishment of both adult and juvenile Chinese mystery snails. The potential to survive in brackish environments widens the range of environments to consider when managing Chinese mystery snails in Nova Scotia. Low pH is likely a limitation for *C. chinensis*, as many juveniles exposed to low pH treatments (initial pH 4.35) did not survive the 28-day period. If the juvenile snails are unsuccessful at survival, population establishment in that environment is unlikely. The pH threshold for *C. chinensis* is still unknown, as there was no significant change in mass or obvious trends in juvenile mortality to suggest a threshold. Further experiments are recommended, potentially over multiple generations, to determine the true threshold for this species. Shoreline surveys indicate that there is a clear seasonal pattern in *C. chinensis* in movements within lakes. We observe significant shifts in shoreline population numbers for three Halifax Regional Municipality lakes, in which the population was found at greater depths with lower water temperature and shallower depths with higher water temperature.

References

Aquatic Invasive Species Task Group, Canadian Council of Fisheries and Aquaculture Ministers. (2004). A Canadian Action Plan to Address the Threat of Aquatic Invasive Species. Retrieved from: https://waves-vagues.dfo-mpo.gc.ca/Library/365581.pdf

Burnett, J. L., Pope, K., L, Wong, A., Allen, C. R., Haak, D. M., Stephen, B. J., & Uden, D. R. (2018). Thermal tolerance limits of the Chinese mystery snail (bellamya chinensis): Implications for management. *American Malacological Bulletin, 36*(1), 140-144.

Chaine, N. M., Allen, C. R., Fricke, K. A., Haak, D. M., Hellman, M. L., Kill, R. A., . . . Vander Ham, A. E. (2012). Population estimate of Chinese mystery snail (bellamya chinensis) in a Nebraska reservoir. *BioInvasions Records, 1*(4), 283-287.

Colautti, R. I., Grigorovich, I. A., & MacIsaac, H. J. (2006). Propagule pressure: A null model for biological invasions; *Biological Invasions, 8*(5), 1023-1037.

Coleman, R. W. (1968). Certain mollusks of the environs of Halifax, Nova Scotia, Canada. *Proceedings of the Iowa Academy of Science, 75*(1), 433-446.

Collas, F. P. L., Breedveld, S. K. D., Matthews, J., van der Velde, G., & Leuven, R. (2017). Invasion biology and risk assessment of the recently introduced Chinese mystery snail, Bellamya (Cipangopaludina) chinensis (gray, 1834), in the Rhine and Meuse river basins in western Europe. *Aquatic Invasions, 12*(3), 275-286.

Crooks, J.A., & Rilov, G. (2009). The Establishment of Invasive Species. In G. Rilov & J.A. Crooks (Eds.), Biological Invasions in Marine EcosystemsL Ecological, Management, and Geographic Perspectives (pp. 173-175). Springer. https://doi.org/10.1007/978-3-540-79236-9_9

Department of Fisheries and Oceans. (2015). Aquatic Invasive Species Regulations. Retrieved from: https://laws-lois.justice.gc.ca/eng/regulations/SOR-2015-121/FullText.html

Department of Fisheries and Oceans. (2019). Aquatic Invasive Species Regulations. Retrieved from: https://www.dfo-mpo.gc.ca/species-especes/ais-eae/regulations-reglements/index-eng.html#enforcement

Escobar, L. E., Mallez, S., McCartney, M., Lee, C., Zielinski, D. P., Ghosal, R., . . . Phelps, N. B. D. (2018). Aquatic invasive species in the Great Lakes region: An overview. *Reviews in Fisheries Science & Aquaculture*, *26*(1), 121-138.

Green, R. (2019). Locals battle smelly snail in lake. *The Laconia Daily Sun*. Retrieved from: https://www.laconiadailysun.com/news/local/locals-battle-smelly-snail-in-lake/article_54340ef4-acc3-11e9-a22d-c76af3cbb46d.html

Haak, D. M., Stephen, B., Kill, R., Smeenk, N., Allen, C.R., Pope, K. (2014). Toxicity of copper sulfate and rotenone to Chinese mystery snails (Bellamya chinensis). *Management of Biological Invasions*. 5: 371-357.

Haak, D. M. (2015). *Bioenergetics and habitat suitability models for the Chinese mystery snail (bellamya chinensis)*.

Haak, D. M., Fath, B. D., Forbes, V. E., Martin, D. R., & Pope, K. L. (2017). Coupling ecological and social network models to assess "transmission" and "contagion" of aquatic invasive species; *Journal of Environmental Management, 190*, 243-251.

Harried, B., Fisher, K., Perez, K. E., & Sandland, G. J. (2015). Assessing infection patterns in Chinese mystery snails from Wisconsin, USA using field and laboratory approaches. *Aquatic Invasions, 10*(2), 169-175.

Havel, J.E. (2011). Survival of the exotic Chinese mystery snail (Cipangopaludina chinensis malleata) during air exposure and implications for overland dispersal by boats. *Hydrobiologia 668: 195-202.*

Hintz, W. D., Schuler, M. S., Jones, D. K., Coldsnow, K. D., Stoler, A. B., & Relyea, R. A. (2019). Nutrients influence the multi-trophic impacts of an invasive species unaffected by native competitors or predators. *Science of the Total Environment, 694*, 133704.

Johnson, P.T.J., Olden, J.D., Solomon, C.T., Vander Zanden, M.J. (2009). Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia* 139: 161-170.

Jokinen, E. H. (1982). Cipangopaludina chinensis (gastropodia: Viviparus chinensis) in North America, review and update. *The Nautilus, 96*(3), 89-96.

Kingsbury, S.E., McAlpine, D.F., Cheng, Y., Parker, E., Campbell, L.M. A review of the non-native Chinese mystery snail, *Cipangopaludina chinensis* (Viviparidae), in

North America, with emphasis on occurrence in Canada and the potential impact on native aquatic species. Unpublished manuscript. Saint Mary's University, Environmental Science.

Latzka, A. W., Crawford, J. T., Koblings, A. S., Caldeira, Y., Hilts, E., & Vander Zanden, M. J. (2014). Representing calcification in distribution models for aquatic invasive species: Surrogates perform as well as $CaCO_3$ saturation state. *Hydrobiologia,*

Leuven, R. S., Boggero, A., Bakker, E. S., Elgin, A. K., & Verreycken, H. (2017). Invasive species in inland waters: From early detection to innovative management approaches. *Aquatic Invasions*, *12*(3), 269-273.

Lodge, D.M., Williams, S., MacIsaac, H.J., Hayes, K.R., Leung, B., Reichard, S., . . . McMichael, A. (2006). Biological invasions: Recommendations for U.S. policy and management. *Ecological Applications*, *16*(6), 2035-2054.

Matthews, J., Collas, F. P. L., de Hoop, L., van der Velde, G., & Leuven, R. (2017). Management approaches for the alien Chinese mystery snail (bellamya chinensis). *Reports Environmental Science, 558*, 1-22

McAlpine, D. F., Lepitzki, D. A. W., Schueler, F. W., McAlpine, F. J. T., Hebda, A., Forsyth, R. G., . . . Noseworthy, R. G. (2016). Occurrence of the Chinese mystery snail, Cipangopaludina chinensis (gray, 1834) (mollusca: Viviparidae) in the Saint John river system, New Brunswick, with review of status in Atlantic Canada. *BioInvasions Records, 5*(3), 149-154.

Museum of Natural History staff (1996). The natural history of Nova Scotia, Volume 1. *Nova Scotia Museum Publications*. Topic 8 (T8): Freshwater. Retrieved from: https://novascotia.ca/nse/surface.water/docs/NSMuseum_FreshwaterEnvironments.pdf

Nova Scotia Lake Chemistry Data (2017). Nova Scotia Lake Survey Program. *Retrieved from: https://novascotia.ca/nse/surface.water/lakesurveyprogram.asp*

Office of the Auditor General of Canada. (2019). Aquatic Invasive Species Report. *Retrieved from*: https://www.oag-bvg.gc.ca/internet/English/parl_cesd_201904_01_43307.html

Olden, J. D., Ray, L., Mims, M. C., & Horner-Devine, M. C. (2013). Filtration rates of the non-native Chinese mystery snail (Bellamya chinensis) and potential impacts on microbial. *Limnetica*, *32*, 107–120. https://doi.org/10.23818/limn.32.11

OATA (2020). New recommendations on buying snails and mussels for garden ponds. *Ornamental Aquatic Trade Association.* Retrieved from: https://ornamentalfish.org/new-recommendations-on-buying-snails-mussels-for-garden-ponds/

Owens, B. (2019). Considering the Nuclear Option in Controlling Invasive Species. *Hakai Magazine: coastal science and societies*. *Retrieved from: https://www.hakaimagazine.com/news/considering-the-nuclear-option-in-controlling-invasive-species/*

Papes, M., Havel, J. E., & Vander Zanden, M. J. (2016). Using maximum entropy to predict the potential distribution of an invasive freshwater snail. *Freshwater Biology, 61*, 457-471.

Ramakrishnan, V. (2007). Salinity, pH, temperature, desiccation and hypoxia tolerance in the invasive freshwater apple snail pomacea insularum. *Dissertations & Theses Europe Full Text: Science & Technology.*

Stanczykowska, A., Magnin, E., & Dumouchel, A. (1971). Etude de trois populations de viviparus malleatus (reeve) (gastropoda, prosobranchia) de la région de Montréal. I. croissance, fécondité, biomasse et production annuelle. *Canadian Journal of Zoology, 49*, 1431-1441.

Stephen, B. J., Allen, C. R., Chaine, N. M., Fricke, K. A., Haak, D. M., Hellman, M. L., . . . Wong, A. (2013). Fecundity of the Chinese mystery snail in a Nebraska reservoir. *Journal of Freshwater Ecology, 28*(3), 439-444.

Strayer, D.L. & Smith, D.R. (2003). A Guide to Sampling Freshwater Mussel Populations. *American Fisheries Society* American Fisheries Society Monograph 8.

Toth, K. (2019). DFO failing in its mandate to control invasive species, auditors find. *CBC News*. Retrieved from: https://www.cbc.ca/news/canada/north/dfo-failing-in-its-mandate-to-control-invasive-species-auditors-find-1.5093140

Underwood, E. B., Darden, T. L., Knott, D. M., & Kingsley-Smith, P. R. (2019). Determining the salinity tolerance of invasive island apple snail pomacea maculata (perry, 1810) hatchlings to assess the invasion probability in estuarine habitats in South Carolina, USA. *Journal of Shellfish Research*, *38*(1), 177-182.

Unstad, K.M., Uden, D.R., Allen, C.R., Chaine, N.M., Haak, D.M., Kill, R.A., Pope, K.L., Stephen, B.J., Wong, A. (2013). Survival and behavior of Chinese mystery snails (Bellamya chinensis) in response to simulated water body drawdowns and extended air exposure. *Management of Biological Invasions* (4)2: 123-127.

Van den Neucker, T., Schildermans, T., & Scheers, K. (2017). The invasive Chinese mystery snail Bellamya chinensis (gastropoda: Viviparidae) expands its European range to Belgium. *Knowledge & Management of Aquatic Ecosystems*, (418), 8.

van Riper, C., Browning, M., Becker, D., Stewart, W., Suski, C., Browning, L., & Golebie, E. (2019). Human-nature relationships and normative beliefs influence behaviors that reduce the spread of aquatic invasive species. *Environmental Management*, *63*(1), 69-79.

Weyhenmeyer, G.A., Hartmann, J., Hessen, D.O., Kopáček, J., Heizlar, J., Jacquet, S., Hamilton, S.K., Verburg, P., Leach, T.H., Schmid, M., Flaim, G., Nõges, T., Nõges, P., Wentzky, V.C., Rogora, M., Rusak, J.A., Kosten, S., Paterson, A.M., Teubner, K., ... Zechmeister, T. (2019). Widespread diminishing anthropogenic effects on calcium in freshwaters. *Scientific Reports*, *9*(1), 10450. https://doi.org/10.1038/s41598-019-46838-w

Waltz, J. (2008). *Chinese mystery snail (bellamya chinensis) review **

Appendix A

Two-way ANOVA test with the independent variables of initial and final masses from each of the five salinity concentration treatments (0 ppt to 25 ppt) for adult and juvenile Chinese mystery snails.

Source	df	MS	F-value	p-value
Adult snails				
Initial vs. final mass	1	133.26	12.174	0.00102
Treatment	4	35.91	3.281	0.01828
Mass : Treatment	4	19.69	1.799	0.14386
Residuals	50	10.95		
Juvenile snails				
Initial vs. final mass	1	0.006120	3.779	0.0545
Treatment	4	0.002080	1.284	0.2807
Mass : Treatment	4	0.001209	0.747	0.5624
Residuals	110	0.001620		

Appendix B

Two-way ANOVA test with independent variables of initial and final masses for each pH treatment (pH 4.35 to pH 7.81) for adult and juvenile Chinese mystery snails.

Source	df	MS	F-value	p-value
Adult snails				
Initial vs. final mass	1	0.37	0.025	0.8749
Treatment	4	35.10	2.406	0.0618
Mass : Treatment	4	0.16	0.011	0.9997
Residuals	50	14.59		
Juvenile snails				
Initial vs. final mass	1	0.000291	0.049	0.8249
Treatment	5	0.02620	4.428	0.000846
Mass : Treatment	5	0.007692	1.300	0.2666
Residuals	156	0.005916		