

The Introduction of *Cipangopaludina chinensis* and Trophic
Effects of Sympatric Freshwater Invaders in Southern Alberta
Reservoirs

by

Megan R. Edgar

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Conservation Biology

Department of Renewable Resources
University of Alberta

© Megan R. Edgar, 2023

Abstract

At present, one of the most pervasive risks to freshwater ecosystems are invasive species. Invasive species are among the leading antagonists to global biodiversity, since they can out-compete or predate on native species which leads to changes of the structure of food webs and alteration of ecosystem function. As biogeographic boundaries are broken down by human-related means of spread, more are we seeing novel occurrences between non-native species and the ecosystems they are introduced to. As invasive species continue to radiate from their original regions, it is critical to understand how these species will impact their new ecosystems. As of 2019, non-native Chinese Mystery Snail (*Cipangopaludina chinensis* (Gray, 1834)) has persisted in McGregor Lake Reservoir, Alberta, Canada. Additionally, Northern Crayfish (*Faxonius virilis*) a non-native species which has expanded their range into lakes and rivers in Alberta, exists in sympatry with Chinese Mystery Snail in McGregor Lake Reservoir.

I aimed to advise management programs related to both Chinese Mystery Snail and Northern Crayfish in Alberta lakes and reservoir systems, and provide important information regarding the trophic interactions and outcomes to native recreational fish species (Northern Pike (*Esox lucius*), Walleye (*Sander vitreus*), and Lake Whitefish (*Coregonus clupeaformis*)).

In this thesis, I provide a comprehensive literature review regarding Chinese Mystery Snail in Canada, along with pertinent information regarding their impacts, life history characteristics, morphological characteristics, and range within invaded and native ranges. The initial finding of Chinese Mystery Snail is officially documented in Alberta, along with biological information that may aid management efforts. DNA barcoding is used to confirm the species identification and the barcode is added to GenBank. We analyze growth rate and fecundity, and assessed the presence of digenean trematode cercariae in snails in McGregor Lake

Reservoir. After a 24-hour experimental period, we confirmed that Chinese Mystery Snail specimens are not infected by digenean trematodes. We also determine that shell growth quickly outpaces shell width, and that upon emergence Chinese Mystery Snail is larger than many of the native snail species in Albertan waterbodies. We also determine that Chinese Mystery Snail in Alberta produce a lower average number of juveniles compared to populations elsewhere. We also suggest that Chinese Mystery Snail meet four of the five stages of species invasion: arrival, establishment, growth, and reproduction, however there is no evidence of dispersal to adjacent waterbodies.

With the use of stable isotope analysis and gut content analysis, I address the trophic consequences that may arise with the addition of Northern Crayfish and Chinese Mystery Snail into aquatic ecosystems in southern Alberta, as well as their impacts to Northern Pike, Walleye and Lake Whitefish. I sampled five reservoirs in Alberta (St. Mary's Reservoir, Forty-Mile Reservoir, Sherburne Lake Reservoir, Travers Lake Reservoir, and McGregor Lake Reservoir), collecting macrophytes, benthic macroinvertebrates, small-bodied fish species, as well as invasives and large recreational fish species. I hypothesized that the presence of only one aquatic invader would create bottom-up effects on the food-web, and that an additional invasive species would amplify these effects, creating shifts in fish trophic positions by moving basal energy away from higher trophic levels through the consumption of algae and macrophytes, or by providing a new prey resource. I also hypothesized that Chinese Mystery Snail and Northern Crayfish would have overlapping niches, through competition for resources or predation. Briefly, I determined that fish are becoming more piscivorous compared to reference lakes where there are no invasive species present, community trophic positions are higher in reservoirs where only Northern Crayfish are present as an invasive, and that carbon ranges are narrower in systems with only Northern Crayfish and wider when both invasives are in sympatry. A few reasons that

these effects may be occurring to fish include an “adaptive lag” period, or lack of macrophytes in invaded waterbodies due to the invasives present. I also discovered that trophic niche ellipses of Chinese Mystery Snail and Northern Crayfish are overlapping, pointing to competitive exclusion.

The results from this thesis address my overall goals to investigate the introduction of Chinese Mystery Snail and the potential impacts of interactions between Chinese Mystery Snail and Northern Crayfish on native recreational fish species in reservoirs in southern Alberta. The findings gained throughout this thesis are useful for the management of both invasive species and will hopefully provide a catalyst for other researchers and resource managers.

Preface

This thesis is an original work by Megan Edgar. Ethics approval for this research project, of which this thesis is a part, was received from the University of Alberta Research Ethics Board, Animal Care and Use Committee “Assessing spread and impact of invasive species” Animal Use Protocol 00003578, April 20, 2020 and provincial Fisheries Research Licence RL 21-2409 granted by Alberta Environment and Parks (AEP). Chapter 3 was partly funded through Alberta Innovates (Canada) #2615 through Patrick C. Hanington.

Co-authors who assisted in the creation and publishing of papers in the following chapters were: Caleb C. Hasler from the University of Winnipeg, Patrick C. Hanington, Robert Lu, Heather Proctor, and Mark S. Poesch from the University of Alberta, Ron Zurawell and Nicole Kimmel from AEP. Contact information is provided in each Chapter preceding the respective paper.

Chapter 2 was written for the Department of Fisheries and Oceans (DFO) following the format for a Biological Synopsis, co-authored with Caleb C. Hasler and Mark S. Poesch. I was responsible for literature review, writing, and editing of this document. Caleb C. Hasler and Mark S. Poesch were responsible for review and guidance.

Chapter 3 has been published as of March 2022 in *BioInvasions Journal*, DOI: 10.3391/bir.2022.11.2.18 as “The first documented occurrence and life history characteristics of the Chinese Mystery Snail, *Cipangopaludina chinensis* (Gray, 1834) (Mollusca: Viviparidae), in Alberta, Canada”. I was responsible for data investigation and collection, result interpretation, writing, reviewing and editing. Patrick C. Hanington worked on investigation and data

collection, data analysis and interpretation, writing, review, and funding provisions. Heather Proctor and Mark S. Poesch worked on reviewing and editing the manuscript. Ron Zurawell worked on field survey, sample collection, and initial review of manuscript. Nicole Kimmel helped with funding provisions, field survey and sample collection. Robert Lu helped with field survey and sample collection.

Dedication

This thesis is dedicated to my parents.

-

“The sentence that best expresses a snail’s way of life: ‘The right thing to do is to do nothing, the place to do it is in a place of concealment and the time to do it as often as possible.’”

- Elisabeth Tova Bailey, *The Sound of a Wild Snail Eating*

Acknowledgements

I would like to begin this section by thanking my thesis advisor Dr. Mark Poesch of the Fisheries and Aquatic Conservation Lab. He consistently allowed this thesis and research to be my own work. Thank you for taking me on as a graduate student in your lab, I have learned a lot in the last two-ish years.

I would also like to thank the experts who were involved in helping me bring everything together, provide academic opportunities, and learn more about the subject matter of invasive species, conservation biology, aquatic and wildlife management, and stable isotopes: Dr. Patrick Hannington, Dr. Caleb Hasler, Dr. Tim Jardine, Dr. Heather Proctor, and from Alberta Environment and Parks; Nicole Kimmel, Emeric Janssens, and Jason Cooper. I would also like to thank the University of Alberta, Nicole Kimmel, and the Alberta Conservation Association (Grants in Biodiversity 2021 program) for funding me throughout my graduate program.

I would also like to acknowledge my lab mates Victoria Van Mierlo, Sebastian Theis, Chloe Christenson, and Jacqueline Pallard who did not just become my friends by being in the same lab together, but were also great brains to bounce ideas off of, learn from, and ask for their opinions and advice about ecology and stable isotope analysis throughout my degree. To my wonderful field technician Kathleen Lalor, thank you for being my friend and forever-snail-hand-model.

I would also like to thank Dr. Lu Carbyn and Dr. Matthew Reudink. Lu, for keeping me thinking about the Big Picture, coffee chats, and birding. And Matt for our great monthly conversations,

academic and professional support, and mostly for being someone that I look up to and keeps me excited about what's to come.

Finally, I have to express my profound gratitude to my parents, family, partner, and friends for providing me with unfailing support and continuous encouragement throughout both my undergraduate degree and my graduate degree, without which I'm sure I would have burned myself out long ago. I would also like to thank my late grandpa, who always encouraged me to treat all animals (people, too!) with compassion. This dissertation stands as a testament to your unconditional love and encouragement.

I would also like to acknowledge that surveys and research for this thesis were carried out on the traditional territories of the Cree, Dene, Blackfoot, Saulteaux, Nakota Sioux, Tsuu T'ina and Stoney Nakoda First Nations, and home of the Métis Nation of Alberta (Treaty 6 and Treaty 7).

Thank you,

Megan Edgar

Table of Contents

<i>Abstract</i>	<i>ii</i>
<i>Preface</i>	<i>v</i>
<i>Dedication</i>	<i>vii</i>
<i>Acknowledgements</i>	<i>viii</i>
<i>List of Tables</i>	<i>xii</i>
<i>List of Figures</i>	<i>xiii</i>
Chapter 1	1
Introduction	1
Chapter 2	4
<i>Biological Synopsis of Chinese Mystery Snail (Cipangopaludina chinensis)</i>	4
Executive Summary	4
Figures	25
Chapter 3	30
<i>The first documented occurrence and life history characteristics of the Chinese Mystery Snail, Cipangopaludina chinensis (Gray, 1834) (Mollusca: Viviparidae), in Alberta, Canada</i>	30
Executive Summary	30
Introduction	31
Material and methods	33
<i>DNA barcoding</i>	34
<i>Phylogenetic analysis</i>	34
<i>Assessment of fecundity and growth</i>	35
<i>Assessment for infection by digenean trematodes</i>	36
Results	36
Discussion	37
Conclusions	40
Acknowledgements	40
Literature Referenced	41
Figures	48
Supplementary Materials	52
Chapter 4	54
<i>Group and community trophic structure within reservoirs in Southern Alberta in the presence of Cipangopaludina chinensis and Faxonius virilis</i>	54
Executive Summary	54
1. Introduction	55
2. Methods	58
2.1. Field sampling	58

2.2. Lab analysis	60
2.3. Statistical Analyses and Modelling	61
3. Results	66
3.1. Catch data	66
3.2. Trophic Position	66
3.3. Comparing community trophic structure and trophic positions	68
3.4. Comparing isotopic niche between <i>C. chinensis</i> and <i>F. virilis</i> in McGregor Lake Reservoir	69
3.5. Mixing Models: Determining diet source proportions of consumers in each reservoir	69
3.6. Gut content analysis.....	71
4. Discussion	71
5. Further comments	75
6. Conclusion.....	77
Tables and Figures	78
Appendix	87
<i>Chapter 5.....</i>	<i>90</i>
<i>General Conclusions</i>	<i>90</i>
<i>Literature Referenced.....</i>	<i>94</i>

List of Tables

Table S3.1: Visual shoreline surveys for *C. chinensis* at seven locations on McGregor Lake (Figure 3.2) and two at Travers Reservoir, conducted by AEP and ASERT in October 2019. Note: Little Bow Reservoir and Little Bow Provincial Park are different. Little Bow Provincial Park is on Travers Reservoir, whereas Little Bow Reservoir is its own waterbody.

Table S3.2: Measurement data collected during dissections of *C. chinensis*, collected in 2020. Samples were collected from McGregor Lake, Alberta.

Table 4.1: List of locations of each reservoir lake, surface area in hectares (ha), mean depth and maximum depth (m)(max.), total phosphorous (TP ($\text{mg}\cdot\text{L}^{-1}/\text{year}$)) and total nitrogen (TN ($\text{mg}\cdot\text{L}^{-1}/\text{year}$)).

Table 4.2: Bayesian estimates of Total Area (TA) (‰^2), Distance to Centroid (CD) (‰), carbon range (CR) (‰) and nitrogen range (NR) (‰) calculated for each system.

Table 4.3: Calculated TA (Total Area) (‰^2), SEA (Standard Ellipse Area) (‰^2), and SEA_c (Standard Ellipse Area corrected for small sample sizes) (‰^2) of *F. virilis* and *C. chinensis* in each reservoir system.

Table A4.1: Summary statistics of each species within each reservoir lake. Reservoirs: MG= McGregor Lake Reservoir, FM= Forty-Mile Coulee Reservoir, SH= Sherburne Lake Reservoir, SM= St. Mary's Reservoir, TR=Travers Reservoir. Group: LKWH = Lake Whitefish, NRPK=Northern Pike, WALL= Walleye, WHSC = White sucker, SPSH= Spottail Shiner, YEPE = Yellow perch.

Table A4.2: Summary table showing the number of individuals of fish, invasive species, invertebrates, macrophyte and periphyton samples collected at each lake. BURB = Burbot, LKWH = Lake Whitefish, LNSC = Longnose Sucker, NRPK = Northern Pike, WALL = Walleye, WHSC = White sucker, YEPE = Yellow perch, SPSH = Spottail Shiner, CMS = Chinese Mystery Snail, NOCR = Northern Crayfish.

Table A4.3: The relative contribution of putative resources to the diet of Lake Whitefish (LKWH), Northern Pike (NRPK), and Walleye (WALL).

List of Figures

Figure 2.1. A) View of operculum on the ventral side of a Chinese Mystery Snail. B) Side view of a Chinese Mystery Snail. Images by Megan Edgar (University of Alberta).

Figure 2.2. Map of countries with reported detections of Chinese Mystery Snail (*Cipangopaludina chinensis*). Countries in red represent invaded countries outside of the native range, whereas countries in green are designated to regions where Chinese Mystery Snails are native.

Figure 2.3. Map of provinces with reported detections of Chinese Mystery Snail (*Cipangopaludina chinensis*).

Figure 2.4. Occurrences of Chinese Mystery Snail (CMS) in western Canada and the United States of America. The map on the left is the extent of southern British Columbia (B.C.). Occurrence dataset points retrieved from GBIF (2021).

Figure 2.5. Occurrences of the Chinese Mystery Snail (CMS) in eastern Canada (Ontario, Quebec, New Brunswick, Nova Scotia), and the United States of America. Occurrence dataset points retrieved from GBIF (2021).

Figure 3.1. Shell of an individual *C. chinensis* from McGregor Lake Reservoir, Alberta, Canada. A. Abapertural view. B. Apertural view. Scale bar = 1 cm. Photo by: Megan R. Edgar.

Figure 3.2. Location of sites surveyed for Chinese Mystery Snail in McGregor Lake and Travers Lake, Alberta, Canada. The inset map shows the survey area relative to British Columbia (B.C.), Saskatchewan (Sask.) and the United States of America boundary. Yellow triangles = *C. chinensis* detected; blue circles = *C. chinensis* not detected.

Figure 3.3. Maximum Likelihood (ML) tree of COI barcodes showing phylogenetic position of *Cipangopaludina chinensis* collected from McGregor Lake Reservoir in southern Alberta,

Figure 3.4. The growth of juvenile *Cipangopaludina chinensis* from McGregor Lake Reservoir, Alberta, Canada. Shell length (dark grey) rapidly outpaces an increase in shell width (light grey) over the first 60 weeks of life. Starting population size of this study was 116 juvenile snails originating from six females. No snails survived to week 60. The shaded areas represent the standard deviation.

Figure 4.1. Map of study locations (McGregor Lake, Travers, St. Mary's, Sherburne Lake, and Forty-Mile Coulee reservoirs) in Alberta (Alta.), Canada. Inset map shows study area (cross-hatched rectangle) relative to Alberta's provincial boundaries shared by British Columbia (B.C.) and Saskatchewan (Sask.).

Figure 4.2: Iso-space plot of means and standard deviations for $\delta^{13}\text{C}_{\text{corr}}$ and baseline calculated $\delta^{15}\text{N}$ for each species within each reservoir. Reservoirs: MG= McGregor Lake Reservoir, FM=

Forty-Mile Coulee Reservoir, SH= Sherburne Lake Reservoir, SM= St. Mary's Reservoir, TR=Travers Reservoir.

Figure 4.3: Box plots of the spread and mean of average trophic position (TP) and $\delta^{13}\text{C}$ in each treatment group, with accompanying pairwise comparisons (SYMP = reservoirs where *F. virilis* and *C. chinensis* are together (sympatry); CONTROL = both species absent (control), St. Mary's Reservoir; NOCR = Only *F. virilis* present).

Figure 4.4: Box and whisker plots showing the spread of $\delta^{13}\text{C}_{\text{corr}}$ (D13Ccorrected) and Trophic position of each focus recreational species (LKWH = Lake Whitefish; NRPK = Northern Pike; WALL = Walleye) in all three treatment groups, with accompanying pairwise comparisons (SYMP = reservoirs where *F. virilis* and *C. chinensis* are together (sympatry); CONTROL = both species absent (control); NOCR = Only *F. virilis* present).

Figure 4.5: Bayesian standard ellipse areas of Northern Crayfish (*F. virilis*) and Chinese Mystery Snail (*C. chinensis*). Dashed lines represent the standard ellipse area and the solid lines represent the standard ellipse area (SEA) corrected for small sample sizes (SEA_c).

Figure 4.6: SIBER Density plot of isotopic niche widths ($\text{SEA}_b\%$) of sympatric *F. virilis* and *C. chinensis* in McGregor Lake Reservoir and *F. virilis* in the reservoirs where they exist allopatrically. The graduations denote 95, 75, 50% credibility intervals with Bayesian estimates. Black dots represent the bootstrapped mean SEA_b areas and the red crosses represent the small sample size corrected standard ellipse area (SEA_c). Reservoirs: MG= McGregor Lake Reservoir, FM= Forty-Mile Coulee Reservoir, SH= Sherburne Lake Reservoir, SM= St. Mary's Reservoir, TR=Travers Reservoir. Species: NC = Northern Crayfish, *F. virilis*, CMS = Chinese Mystery Snail, *C. chinensis*.

Chapter 1

Introduction

Freshwater ecosystems are constantly experiencing changes, although these changes are now becoming novel, due to multiple human-induced consequences that we have not yet experienced, and exceptionally large (Carpenter et al. 2011). These changes are creating abnormal levels, relative to recent decades, of impacts due to human transformation of inland freshwater ecosystems, such as rivers, lakes, reservoirs, wetlands, and ponds. Many of these changes due to the high levels of human disturbance contribute to the growing frequency of extinction amongst freshwater species (Nicacio and Juen 2015). Some of the drivers for these changes include the climate crisis, anthropogenic alterations of water flow, land-use and cover, and chemical inputs.

One of the most pervasive risks to aquatic ecosystems are invasive species. Invasive species are among the leading antagonists to biodiversity globally; as invasive species become established, they can out-compete or predate on native species, changing food web structure and altering ecosystem function (Dextrase and Mandrak, 2006; Olden et al, 2009). Invasive species are those that have been translocated long distances from the species' native ranges and introduced into new environments, which then have adverse effects on native species. These biogeographic barriers have been expertly broken down by humans through the enabling of international travel, thus intentionally or unintentionally transporting these invasive species to new regions. A larger proportion of introduced species fail to establish but in freshwater ecosystems many have become invasive and caused issues for native aquatic species (Havel et al. 2015).

Despite an increase in the amount of literature regarding invasion effects, many of the impacts remain unstudied, especially the effects of multiple invasives together (Ruiz et al. 1999; Ross et al. 2004; Simberloff and Holle). Invaders can negatively affect one another through competition and predation, reducing one another or minimizing their joint impacts on native biota (Ross et al. 2004). Invasive species may have no effect on each other, or they may have a facilitative interaction, increasing ecological impacts and benefiting establishment and spread (Ricciardi 2001; O'Dowd et al. 2003). Some studies have suggested that once a non-native species has become established, they can alter the ecosystem to facilitate future invasions, described as the invasional meltdown hypothesis (Ricciardi, 2001). This hypothesis, according to Ricciardi (2001), postulates that ecosystems are more easily invaded as the cumulative number of non-native species increases, and that mutualistic and commensal interactions may exacerbate the effects of non-native species. Though there have been many studies on the effects of single invasive species, there are less studies on the effects of more than one invasive species. As observed in a literature review by Simberloff and Holle (1999), any sort of mutual interactions between introduced species are less likely to be sought out and reported, than the effects of introduced species on native ones. For many researchers, conservationists and resource managers, the penultimate concern is the potential damage that non-natives will inflict on native species. However, the extent that facilitative interactions among non-native species, at least some of them will have negative effects on native species.

There is a continuous need to understand how invasive species will impact the ecosystems that they are introduced to. Without this understanding, managers and conservationists alike would not know the level to which they should be interfering with their

removal or how to best manage the invasive species. Of course, mitigating the introduction is priority, but if research is done and it is determined that there are minimal impacts then funds can be allocated to alternative conservation measures.

In this thesis, an important investigation into the first introduction of Chinese Mystery Snail (*Cipangopaludina [=Bellamya] chinensis* (Gray, 1834)) in Alberta is discussed, and a look into the effects that two sympatric invaders may have on aquatic ecosystems. The research herein addresses my overall goal to investigate the novel introduction of *C. chinensis* and the potential impacts of *C. chinensis* and *F. virilis* on native recreational fish species in reservoir ecosystems in southern Alberta. The findings gained throughout these studies are useful for the management of *C. chinensis* and *F. virilis* in southern Alberta, hopefully providing a catalyst for other researchers and resource managers.

Chapter 2

Biological Synopsis of Chinese Mystery Snail (*Cipangopaludina chinensis*)

Executive Summary

The Chinese Mystery Snail, *Cipangopaludina chinensis*, is a recently introduced aquatic invasive species that may threaten Canadian lotic and lentic freshwater ecosystems. This biological synopsis summarizes information regarding the species' description, distribution, biology, natural history, and its use by humans and impacts. The purpose of this synopsis is to be used to estimate the risk of invasion of the Chinese Mystery Snail in Canada. The Chinese Mystery Snail is native to Asia, including China, Taiwan, Korea, and Japan. It was first introduced to North America through food markets in San Francisco, California, United States of America in the 1890s, preceding the spread through the mid-continental USA, where Chinese Mystery Snail established in 34 states. It was first reported on the east coast of Canada in St. John's, Newfoundland and Labrador in 1905 and has subsequently been reported in Ontario, Nova Scotia, New Brunswick, British Columbia, and most recently, Alberta. There are also 12 known isolated populations, as of 2007, with one successfully established population in the Netherlands. As of 2016, the Chinese Mystery Snail has also successfully established one population in Belgium. The Chinese Mystery Snail is dispersed via human-mediated transports, such as release from aquariums, recreational activities, through adhesion to watercraft, as well as the potential transport by wading birds and waterfowl. They can change food web dynamics, nitrogen and phosphorus levels in the water column and may lead to infrastructure damage. The Chinese Mystery Snail has been documented to amplify other aquatic invasive species' impacts when present in the same ecosystem. Due to the operculum present on the Chinese Mystery Snail, its ability to survive desiccation for up to nine weeks, and multiple reports of establishment in a

multitude of slow-moving water, the presence of Chinese Mystery Snail is of great concern to biodiversity in Canadian water bodies.

1. Introduction

Global biodiversity is largely affected by aquatic invasive species, and though large-scale invasion events are rare, introductory events are on the rise (David and Cote, 2019; Levine and D'Antonio, 2003). The first reported occurrence of Chinese Mystery Snail, *Cipangopaludina chinensis*, in North America was in San Francisco, California, via Asian food markets in the 1890s (Wood, 1892; Haak, 2015). Chinese Mystery Snail, is a relatively new invasive species in Canada, with the first occurrence in St. John's, Newfoundland, in 1905 (Kingsbury et al., 2020). Since then, Chinese Mystery Snails have proliferated in many introduced lentic and slow-moving lotic water bodies in the mid-continental United States of America, and have spread across Canada (GISD, 2017; Kingsbury et al., 2020).

The Chinese Mystery Snail is difficult to eradicate once established due to their distinctive operculum or “trap-door”, which closes in times of environmental disturbance or threat. Thus, the operculum shelters the species from desiccation, thermal stressors, or chemical means of population control (Unstad et al., 2013). Chinese Mystery Snail may also interact with other species in non-indigenous ranges, including other invasive species (i.e., invasional meltdown theory; Johnson et al., 2009; Ricciardi, 2001), and species at risk (Kingsbury et al., 2021).

Repeated observations of Chinese Mystery Snail in Canada, coupled with the predicted rise in global temperatures and species fecundity and adaptability, have led to concerns that the species might establish itself and spread northward in Canada (Kingsbury et al., 2020). Therefore, a biological synopsis of the Chinese Mystery Snail is presented here as a prerequisite for a risk assessment of this nonindigenous species in Canadian aquatic systems.

1.1.Name And Classification

From the Integrated Taxonomic Information System (ITIS), 2007, and the Invasive Species

Specialist Group (ISSG), 2006:

Kingdom: Animalia

Superphylum: Lophozoa

Phylum: Mollusca

Class: Gastropoda

Subclass: Prosobranchia (Milne-Edwards, 1848)

Order: Architaenioglossa

Family: Viviparidae (Gray, 1847)

Subfamily: Bellamyinae (Rohrbach, 1937)

Genus: *Cipangopaludina* (Hannibal, 1912)

Species: *Cipangopaludina chinensis* (Gray, 1834)

Other scientific name: *Cipangopaludina chinensis chinensis* (Gray, 1834), *Cipangopaludina chinensis malleata* (Reeve, 1863), *Bellamyia chinensis* (Smith, 2000)

Common English name: Chinese Mystery Snail

Other English name: Asian apple snail, Asian freshwater snail, Chinese mysterysnail, mystery snail, Oriental mystery snail, trapdoor snail, Chinese trapdoor snail, Oriental mystery mollusk, Asian mud snail

Common French name: *Vivipare orientale*

1.2. Species Description

Chinese Mystery Snail, *Cipangopaludina chinensis*, is a benthic grazer and filter-feeder, a dioecious gastropod that primarily feeds on benthic and epiphytic diatoms (Plinski et al., 1977).

It is the second largest snail in North America, with some individuals reaching a length of 65 mm (Pace, 1973). Chinese Mystery Snails vary in colouration between juvenile and adult life stages. As a juvenile, the Chinese Mystery Snail is lighter in coloration than its adult life stage, which can be olive green, greenish-brown, reddish-brown, or brown (Clarke, 1981; Jokinen, 1992). Chinese Mystery Snail has a thick shell and “trap-door” operculum (Figure 2.1A), which allows isolation from external conditions when environmental conditions are unfavorable

(Solomon et al., 2010). As adults, their globose shells, with concentrically marked opercula (Burch, 1980), have between 6 to 7 whorls with a width to height ratio of 0.74–0.82 (Clarke, 1981; Jokinen, 1992). Juvenile shells differ in that their last shell whorl has a distinct carina, and will have 20 striae per mm between each groove (Clarke, 1981; Smith, 2000). Juvenile snails also have a detailed pattern on their periostracum, consisting of 2 apical and 3 body whorl rows of hairs with long hooks on the end, as well as distinct ridges and many additional hairs with short hooks (Jokinen, 1984). Another identifying feature in Chinese Mystery Snails is that they have a small, round umbilicus, with a spire at an angle between 65° and 80° (Jokinen, 1992).

The Chinese Mystery Snail is often confused with Japanese mystery snail, *Cipangopaludina japonica*. Though the closely related snails are similar, the Chinese Mystery Snail's shell grows allometrically, with the shell height increasing faster than the shell width (Jokinen, 1982), which is a unique characteristic. In the past, the radula has been used to differentiate the two species, though there may not be enough variation between the two to make this a good diagnostic characteristic (Smith, 2000). In a North American population of Chinese Mystery Snail the radula has seven small cusps on the marginal tooth and a large central cusp with four small cusps on either side (Jokinen, 1982).

1.3. Taxonomy of *Cipangopaludina chinensis*

The taxonomy of *Cipangopaludina chinensis* has often been contested, with multiple scientific names in use. *C. chinensis* is sometimes referred to as *Bellamyia chinensis* (Smith, 2000) in literature (Kelley, 2016), nevertheless *B. chinensis* does not have official recognition (ITIS, 2016). There has also been debate regarding *C. chinensis* and *C. japonica* and whether or not they are synonymous species in North America, with different phenotypic variations. David and

Cote (2019) conducted a genetic and morphological analysis of both species' populations in North America and determined that the species were genetically distinct, despite indistinguishable morphological features. For the purpose of this biological synopsis, *C. chinensis* and *C. japonica* will be considered as two individual species.

2. Distribution

2.1. Native Distribution

The Chinese Mystery Snail is native to Asia, including China, Taiwan, Korea, Japan, Indonesia, Burma, the Philippines, Asiatic Russia in the Amur Region, Thailand and South Vietnam (Pace, 1973; Chiu et al., 2002; GISD, 2017). Others have noted that the risk of potentially misidentifying *C. chinensis* within its native range is high due to the presence of visually similar species, thus reducing the certainty of its range (Matthews et al., 2017). That being said, its presence is most often reported in China, Taiwan, Korea and Japan (Lu et al., 2014). In China and other countries within its native distribution, the Chinese Mystery Snail can be found in pools, lakes, streams, and rice paddy fields (Li, 2012).

Introduced Range

2.1.1. The United States of America (North America)

The Chinese Mystery Snail was first introduced through food markets in San Francisco, California, in the 1890s (Wood, 1892; Haak, 2015). Chinese Mystery Snail subsequently spread to 34 other states in the United States, including Alaska and Hawaii (GISD, 2017; Havel, 2011), notably in lentic and slow-moving lotic habitats (Distler, 2003). They have been reported in Massachusetts and are thought to have been introduced accidentally along with goldfish (*Carassius auratus*), which were being used for mosquito control (Distler, 2003). The Chinese

Mystery Snail has been documented in Maine, south to Florida, and mid-continentally from the Great Lakes, Iowa, Nebraska, Oklahoma, Texas, Colorado and Utah (Distler, 2003). In the early 1900s, the Chinese Mystery Snail was also introduced to the Hawaiian island as a food item by Chinese immigrants (Cowie, 1998).

According to Solomon et al. (2010), Chinese Mystery Snails are most commonly abundant in the southern regions of their invaded ranges in North America. Though with predicted increase in global temperatures in the future due to climate change, this may change, increasing the likelihood for Chinese Mystery Snail to disperse northward in introduced ranges (Kingsbury et al., 2020).

2.1.2. European Union Member States

The Chinese Mystery Snail was first observed in the Netherlands in 2007 (Collas et al., 2017). There are now 12 isolated populations, which have been established for several years at three locations. The Chinese Mystery Snail was also observed in Belgium, as recently as 2016, with one established population (Matthews et al., 2017; Collas et al., 2017; Van den Neucker et al., 2017).

2.2. Records In Canada

The first known occurrence of Chinese Mystery Snail in Canada was in St. John's, Newfoundland in 1905 (Kingsbury et al., 2020). Since then, the Chinese Mystery Snail has established in southern Ontario, including Lake Erie and embayments of western Lake Ontario, Kawartha Lakes, and Crower, Moira and Trent River drainages (Therriault and Kott, 2002). According to a review conducted by Kingsbury et al. (2020), there have most recently been 278 reports documenting the presence of Chinese Mystery Snail in Canada, with the largest reports originating from Ontario (Figure 2.3; Figure 2.4; Figure 2.5).

The Chinese Mystery Snail has also been reported in Québec (Clarke, 1981; Tornimbeni et al., 2014) and British Columbia (Clarke, 1981), specifically in five lakes on Southern Vancouver Island, four reports from downtown Victoria, and one confirmed report in the town of Mission (Figure 2.4) (Government of British Columbia, n.d.). Chinese Mystery Snail has also been reported in Atlantic Canada, from sites in Nova Scotia, New Brunswick and Newfoundland (Figure 2.5) (McAlpine et al., 2016). The Chinese Mystery Snail was recently reported in 2019 in McGregor Lake, near the Village of Milo (Alberta Environment and Parks, 2019; Mitchell et al., 1990). Due to their very recent detection, little is known about their range dispersal or population estimates in Alberta. Before their initial detection, in 2015, Chinese Mystery Snail was listed under the Fisheries Act of Alberta as a prohibited species, making it illegal to possess, release, sell or transport within the province (AEP, 2019). Chinese Mystery Snail is also listed in the Water Protection Act of Manitoba (Government of Manitoba, 2015).

Of these reports, the Chinese Mystery Snail has successfully established populations in 146 locations across Canada. The most reported occurrences were in the following habitats: 64 lakes, 29 rivers and 25 ponds, however there were also reports from 1 marsh, 5 bays, and a harbor (Kingsbury et al., 2020).

2.3.Potential Distribution in Canada

Previous research in North America has suggested that Chinese Mystery Snail may prefer lentic, slow-moving water bodies (McAlpine et al., 2016). Water chemistry and ecosystem composition help determine where they may successfully establish when introduced through, largely, human-

mediated transfers (Bury et al., 2007; Haak, 2015; Jokinen, 1982; Kingsbury et al., 2020; Kingsbury et al., 2021).

Solomon et al. (2010) has also stipulated that shoreline development, distance to population centres, presence of boat landings, species composition of water bodies, Secchi depth, conductivity, and calcium concentrations are critical factors in determining appropriate habitat.

According to studies conducted by Kingsbury et al. (2021), using random forest models (RFMs) and habitat suitability models, the critical predictive parameters for likely habitat depends on the number of fish species present, alkalinity, presence of other invasive species, number of accessible boat launches, and connected freshwater bodies. Though other parameters may be used, such as latitude and longitude, the datasets are skewed towards the high proportion of Ontario reports (Kingsbury et al., 2021).

2.4. Population Genetics

Using molecular barcoding analyses, David and Cote (2019) successfully characterized the difference between Chinese Mystery Snail, *Cipangopaludina chinensis*, and Japanese mystery snail, *C. japonica*. Before this, there was discordance regarding the difference between the two, as there is little morphological difference, save for a taller conchological spire on *C. japonica* (David and Cote, 2019).

Phylogenomic analyses conducted by Hirano et al. (2019) focused on the two subspecies of Chinese Mystery Snail, *Cipangopaludina chinensis*: *C. chinensis chinensis* (Gray, 1834) and *C. chinensis laeta* (Martens, 1860). *C. C. chinensis* is found within mainland Asia, including

China and Vietnam, whereas *C. c. laeta* is located in Japan (Hirano et al., 2019). This research discovered that the species diverged in the Pliocene to early Pleistocene era, through introgressive hybridization with now extinct species or population (Hiran et al., 2019). The subspecies are capable of crossbreeding. In the case of crossbreeding, a female *C. c. laeta* and male *C. C. chinensis* may only have *C. c. laeta* juveniles, while contrary, *C.C. chinensis* may not have *C. c. laeta* juveniles (Hirano et al., 2019).

2.5. Pathways Of Introduction and Transfer

Live Chinese Mystery Snails were initially introduced in North America in 1891, from Japan to the Chinese food markets in San Francisco, California (Pace, 1973). In 1894, three years after the first initial report of Chinese Mystery Snail in North America, it was reported that they were also sold in a food market in Victoria, British Columbia (Jokinen, 1982). A common opinion is that the Chinese Mystery Snail was initially brought to the United States and Canada from China, via immigration, and subsequently released into local waterways as a new food source (Jokinen, 1982). Snails are also often released from the aquarium and ornamental trade into the wild, whether deliberately or accidentally (Collas et al., 2017).

Bury et al. (2007) stated that after the initial release and establishment of Chinese Mystery Snails in lakes, there is a potential for spread to other water bodies via rivers due to upstream lake populations serving as sources for downstream colonization.

The risk of snails invading new water bodies frequently relies on their ability to survive overland transport through various means (Havel et al., 2014). Recreational boating constitutes the main vector for overland transportation of aquatic invasive species, including macrophytes

and molluscs and, notably, invasive snails (Havel et al., 2014; Johnson et al., 2009; McAlpine et al., 2016). Invasive snails may get entangled in macrophytes, which may get sucked into propellers and entangled on boats and trailers (Jokinen et al., 1982). The potential vector for spread, through boat and trailer, is only amplified in the summers, when Chinese Mystery Snails move into shallow water (Havel et al., 2014). The Chinese Mystery Snail can survive multiple weeks of desiccation, thus increasing their threat of invasion through adhering to and hitchhiking on boats (Havel et al., 2014).

Another pathway of introduction that should be considered, though less studied and anecdotal, is introduction via transportation of waterfowl and wading birds. In a study conducted by van Leeuwen and van der Velde (2012), aquatic snails' potential transport via ectozoochory, external transport by adhering to waterfowl, was investigated. Though their research suggested that dispersal by this method may only be relevant to short-distance dispersal, this is enough to transport aquatic invasive snails, the Chinese Mystery Snail, to adjacent water bodies. Dispersal by waterfowl may be an explanation for the generally expeditious colonization of lentic water bodies and the widespread distribution of aquatic snails (van Leeuwen and van der Velde, 2012).

3. Biology and Natural History

3.1. Life Cycle

Chinese Mystery Snails are gonochoristic and ovoviviparous, releasing fully formed young (Haak 2015, 2009; Havel, 2014). Female Chinese Mystery Snails give live birth, releasing live young between May and through October (Havel, 2011; Haak, 2015).

Chinese Mystery Snails continue to grow throughout their entire lifespan. Female snails are reported to survive a maximum of five years, and males survive four years (Jokinen, 1982). Consequently, females are generally larger than males. As Chinese Mystery Snails age, their shell grows to be more elongated than they are high; the shell width ratio to shell height decreases as the shell increases in size (Jokinen, 1982).

Chinese Mystery Snails are viviparous with an iteroparous reproduction strategy; females produce young nearly every year until death (Stephen et al., 2013). They are able to produce young within their first year until they reach a maximum age of five years (Stańczykowska et al., 1971). Studies have reported that Chinese Mystery Snails average approximately 25 juveniles per female, with an annual fecundity between 27.2 and 33.3 young per female per year (Stephen et al., 2013). Their reproductive pattern and the number of average young per female suggest that explosive population growth may be possible in regions with newly introduced Chinese Mystery Snail (Stephen et al., 2013).

3.2. Physiological Tolerances and Requirements

The Chinese Mystery Snail's shell and operculum allow them to be resistant to desiccation and long periods kept out of water. Larger individuals have been documented to survive for more than nine weeks out of water in laboratory experiments (Unstad et al., 2013). Others have reported that Chinese Mystery Snails require high parts per million of calcium in water and that they are intolerant of and incapable of growth in soft water (Jokinen, 1982). Calcium is required for the growth of snails, especially for aquatic snails, and may even be more overriding than having dietary sources available (Plinski et al., 1977). A laboratory study conducted by Burnett et al. (2018), identified an upper thermal tolerance of 45° Celsius. They did not identify a lower

thermal tolerance, which suggests that the lower lethal thermal tolerance was less than 0° degrees Celsius. With this information, it can be conveyed that Chinese Mystery Snails are a temperature hardy species and that it has a higher chance of surviving winters and benthic temperatures in most water bodies of Canada (Wetzel, 2001).

3.3. Behaviour And Movements

When an aquatic snail with an operculum moves or crawls, the operculum is moved to the outside not to hinder movement (Bhushan, 2009; Xu et al., 2018). Following many other species, the Chinese Mystery Snail has evolved to have a non-smooth surface on the operculum, for drag reduction and to resist wear, creating a tool for hydrodynamic lubrication (Xu et al., 2018). According to further movement studies conducted by Xu et al. (2018), they concluded that the Chinese Mystery Snail, compared to other aquatic snails (*Pomacea canaliculata* and *Pomacea bridgesii*), are far less active than other snails and, instead, bury themselves in muddy or silty bottoms. This was discovered through studies of friction and velocity calculations based on morphological characteristics, such as shell thickness, grooves, ridges, and surface roughness of the operculum. In the study, the Chinese Mystery Snail had a friction coefficient ten times larger than *P. canaliculata*, another water snail species (Xu et al., 2018).

Chinese Mystery Snail is not reported to exhibit predator avoidance behaviour or move when there are predators, such as crayfish. Instead, they stay attached to substrate or they will stay at the bottom of a waterbody with their opercula shut (Kelley, 2016).

3.4. Habitats

As reported by others, the Chinese Mystery Snails prefer lentic water bodies with silt, sand, and mud substrates in eastern North America (Jokinen, 1982; Stańczykowska et al., 1971), although it has been documented to survive in lotic freshwater, notably slower streams (Distler, 2003; Jokinen, 1982). Other documentation observed populations living on artificial substrates, riprap, and submerged vegetation (Chaine et al., 2012). The Chinese Mystery Snail is able to tolerate stagnant waters near septic tanks (Perron and Probert, 1973). The Chinese Mystery Snail has been documented in water bodies with a pH level between 6.5 and 8.4, and various concentrations of calcium, magnesium, oxygen, and sodium; 5 - 97 ppm, 13 - 31 ppm, and 7 - 11 ppm, 2 - 49 ppm, respectively (Jokinen, 1982; Jokinen, 1992; Stańczykowska et al., 1971). It is most commonly found at depths between 0.2 and 3 metres, with a conductivity of 63 - 400 µmhos/cm (Jokinen, 1982; Jokinen, 1992; Stańczykowska et al., 1971).

3.5. Feeding and Diet

At each life stage, the Chinese Mystery Snail non-selectively grazes on periphyton (Johnson et al., 2009) and non-organic bottom material, such as benthic and epiphytic algae (Jokinen, 1982). Older, larger individuals are also capable of filter-feeding (Olden et al., 2013). Distler's (2003) gut-content analysis suggests that they radular feed by scraping on inorganic and organic debris with their radula, and on epiphytic-benthic algae, primarily diatoms.

3.6. Interspecific Interactions

Chinese Mystery Snails have been documented to interact with other invasive species, such as Rusty Crayfish (*Orconectes rusticus*), native crayfish, as well as native snail species (Johnson et al., 2009; Kelley, 2016). Mesocosm and lab studies have shown that Rusty crayfish can consume

Chinese Mystery Snails, suggesting that the two invasives may interact in locations where they have both been established (Johnson et al., 2009). Interactions between Chinese Mystery Snails, other snails, and natural predators in the same ecosystem, such as crayfish, are influenced mainly by snails' shell thickness (Kelley, 2016). Kelley (2016) reported that crayfish rarely ate Chinese Mystery Snails that were beyond the size of 20 mm, though they do reach a maximum shell length of 60 mm. Olden et al. (2009) reported that smaller Chinese Mystery Snails were more likely to be consumed by crayfish, though larger individuals were also susceptible to predation.

Snails of larger sizes are more likely to avoid predation by crayfish due to shell thickness, which gets thicker as snails age (Kelley, 2016). Kelley (2016) and Olden et al. (2009) concur that Chinese Mystery Snails have a high susceptibility to crayfish predation, which might be a large limiting factor in their population growth and dispersal. This may depend on the morphological characteristics of native snails sharing the same ecosystem, as some native snails may be smaller and thus incite a shift away from crayfish predation on Chinese Mystery Snails.

3.7. Parasitic Infections and Diseases

Chinese Mystery Snail is a host for several helminth parasites that affect humans in its native range of Asia. Therefore, it may be a vector for parasites and diseases, such as human intestinal fluke, in invaded ranges (Chung and Jung, 1999; Havel, 2011). Though this may be the case, there is little data to support this, and there have been no reported cases of human intestinal fluke transmitted by Chinese Mystery Snail in the United States (Bury et al., 2007).

According to recent research conducted by Harried et al., results suggested that there are relatively low trematode infection levels in invaded ranges (2015). These results contrast the

infection rates of parasites in Chinese Mystery Snail in native ranges. Though there is a general lack of parasite infection of Chinese Mystery Snail in invaded ranges, this may present additional consequences for establishing and dispersal of Chinese Mystery Snails in non-native ranges (Harried et al., 2015). This is because infected snails generally produce less offspring and lessen the probability of survival (Harried et al., 2015; Sandland and Minchella, 2003). Therefore, individuals in their invaded range may have stronger fitness advantage when compared to individuals from their native range (Harried et al., 2015). However, the study found two helminth parasites within two individual Chinese Mystery Snails (Harried et al., 2015). The first of the parasites found was *Cyathocotyle bushiensis*, a species that causes mortality of dabbling ducks (Harried et al., 2015; Hoeve and Scott, 1988; Kingsbury et al., 2020). The second parasite was *Aspindogaster conchicola* (Harried et al., 2015; Kingsbury et al., 2020), a parasite that affects freshwater bivalves, fish and turtles in North America, Africa and Europe (Kingsbury et al., 2020). The individual snails with these parasites only presented metacercarial cysts encased within the mollusc shell, and thus were not likely viable (Harried et al., 2015), nevertheless it is concerning that an individual Chinese Mystery Snail was found hosting *A. conchicola*, as this species has been cause for declines in successful reproduction of North American freshwater mussels (Gangloff et al., 2008; Kingsbury et al., 2020).

4. Human Uses

4.1. Uses As a Human Food

The Chinese Mystery Snail is edible and sold in Asian food markets in the United States (Benson, 2007). Though the risk of parasite transmission and gap in knowledge concerning Chinese Mystery Snails as a food source in Canada, it cannot be guaranteed as safe. In native ranges, the Chinese Mystery Snail serves as a host for several helminth parasites that affect

humans, including human intestinal fluke (Chung and Jung, 1999). As is currently the case, there have been no reported cases of humans contracting intestinal fluke in the United States of America through consumption of Chinese Mystery Snail (Bury et al., 2007).

4.2. Other Uses

In Asian countries, a variety of different uses of Chinese Mystery Snail have been documented. Contrasting its potential impacts in North America and other invaded ranges, the Chinese Mystery Snail is an essential bioindicator in Asia (Li, 2012) and represents ideal, healthy freshwater systems (Li, 2012, Q.H. Gu et al., 2015). Chinese Mystery Snail may be useful in removing sewage contaminants and heavy metals found in rice paddy soil (Kurihara and Suzuki, 1987). Further, Chinese Mystery Snail is often used in restoration projects involving engineered wetlands, in an effort to increase water clarity and restore natural aquatic ecosystems (Li, 2012). Chinese Mystery Snail is also used as feed for larval fireflies, *Luciola ficta* (Ho et al., 2010; Kingsbury et al., 2020), and hatchery-cultured mud crab, *Scylla paramamosain*, (Gong et al., 2017; Kingsbury et al., 2020). According to Yan (2002), Chinese fish farms often use viviparid snails as commercial food for Chinese carp, *Mylopharyngodon piceus* and *Cyprinus rubrofasciatus*, as well as for softshell turtles (Tian et al., 2012) and Chinese gold-coin turtles (*Cuora trifasciata*) (Kingsbury et al., 2020; Luo et al., 2012).

Chinese Mystery Snail has long been used as traditional Korean medicine and a part of Korean medicinal knowledge to cure indigestion (Kim et al., 2018; Kingsbury et al., 2020). There have also been further efforts to develop a pharmaceutical use for Chinese Mystery Snail in Asia, in an effort to explore the efficacy of compounds derived from Chinese Mystery Snails to protect against liver damage (Kingsbury et al., 2020; Wang et al., 2015; Xiong et al., 2019),

joint pain caused by immune responses (Lee et al., 1988; Shi et al., 2016), and to help prevent plaque build-up in arteries (Kingsbury et al., 2020; Xiong et al., 2019). Calcium has also been recycled from waste shells in an attempt to promote bone growth (Zhou et al., 2016). According to Kingsbury et al (2020), these methodologies could potentially transfer to North America, and eventually be used as a solution to slow the spread and population growth of invasive Chinese Mystery Snail.

5. Impacts Associated with Introductions

Although there have been several recorded incidences of Chinese Mystery Snail in Canada, there is not much information regarding their impacts as they are relatively new introduced species. Much of what is known about Chinese Mystery Snail can be used to hypothesize their impact on environments in Canada.

5.1. Ecological Interactions

The Chinese Mystery Snail has been known to interact with other species, though due to their relatively new arrival in introduced regions, there is a gap in knowledge regarding these interactions (Haak, 2015). Previous mesocosm and lab studies have suggested that when Chinese Mystery Snail is coupled with Rusty crayfish (*Orconectes rusticus*) in the same ecosystem, the indigenous snail communities (*Physa*, *Lymnaea*, and *Heliosoma* spp.) will be negatively affected (Johnson et al., 2009). Both *Physa* and *Lymnaea* species have thinner shells, and are thus easier to consume than the thick shelled Chinese Mystery Snail. *Heliosoma* species may afford greater protection from fish and crayfish species as they have a thicker shell (Johnson et al., 2009). Consequently, if Chinese Mystery Snail has a competitive advantage against indigenous snails,

with a thicker shell, in the presence of crayfish or predatory fish, this may lead to declines in indigenous snail species (Johnson et al., 2009; Rundle and Brönmark, 2001).

Another mesocosm study in Washington suggested that the presence of Chinese Mystery Snail may have exacerbated the abundance of invasive Northern Crayfish (*Faxonius virilis*) by providing an abundant prey source (Johnson et al., 2009).

Chinese Mystery Snail grazing can also lead to reduced algal biomass, altered algal species composition, increased nitrogen to phosphorus ratio in the water column, elevated eutrophication levels, and altered food webs through changes in bacterial community composition (Kingsbury et al., 2021). Grazing by Chinese Mystery Snail has been observed to negatively affect periphyton levels, notably within an ecosystem's sediment. The ratio of total nitrogen and phosphorus within a mesocosm experiment increased in the presence of Chinese Mystery Snail (Johnson et al., 2009). This may be explained by a low level of phosphorus excreted, compared to other snails, thus creating a phosphorus "sink", reducing its availability in the environment (Johnson et al., 2009). These changes could cause significant adverse impacts to other species that use these aquatic ecosystems, including Atlantic whitefish and brook floater mussel, as well as many other species at risk that are already threatened by other natural disturbances (COSEWIC, 2009; Fisheries and Oceans Canada, 2018; Kingsbury et al., 2021).

5.2. Impacts On Habitat Integrity

Chinese Mystery Snails have been shown to cause changes to algal communities, and nitrogen and phosphorus ratios in the water column. In an anthropogenic dynamic, they have the potential

to damage human infrastructure, such as drainage and reservoirs, and be the cause of windrows of shells on shorelines (Kingsbury et al., 2021).

Previous mesocosm studies have demonstrated that the Chinese Mystery Snail causes significant increases in the molar ratio of phosphorus and nitrogen in aquatic ecosystems (Johnson et al., 2009). They also influence algal growth in mesocosms by decreasing sediment periphyton and benthic algae, thus decreasing levels of chlorophyll a and altering benthic algal communities (Johnson et al., 2009; Kingsbury et al., 2021; Mo et al., 2017).

6. Summary

This compilation of information regarding the ecology, distribution, life history and prospective impacts of the Chinese Mystery Snail may be used to assess its further distribution, establishment, and impacts to Canadian aquatic ecosystems. With the first occurrence of Chinese Mystery Snail within Canada in St. John's, Newfoundland in 1905, the species has made its way through the southern parts of many provinces, west to British Columbia (Kingsbury et al., 2020). The main pathways of distribution are considered to be human-mediated transport via adhesion to boat hulls, recreational activities, pet-trade, and potentially by waterfowl. The species is difficult to eradicate, as a consequence of its operculum, which seals the shell when threatened or in the presence of ecological disturbance, making this species resilient to desiccation for up to nine weeks (Unstad et al., 2013), heat (Burnett et al., 2018) and chemical management (Haak, 2014; Haak, 2015). The Chinese Mystery Snail cannot be effectively managed through the use of culling methods or drawdowns (Unstad et al., 2013). Chinese Mystery Snail may disrupt nitrogen and phosphorus levels in aquatic ecosystems, thus increasing the likelihood of eutrophication in water bodies where they have successfully established (Kingsbury et al., 2021;

Mo et al.,2017). They may also alter food web interactions and trophic systems, through competition with indigenous snail species and potentially other invasive species in the same waterbody, as demonstrated in studies involving both Rusty crayfish and Chinese Mystery Snail (Johnson et al., 2009). Interactions between Chinese Mystery Snail and an additional invasive species may have additive consequences or be facilitative (Johnson et al., 2009).

Figures

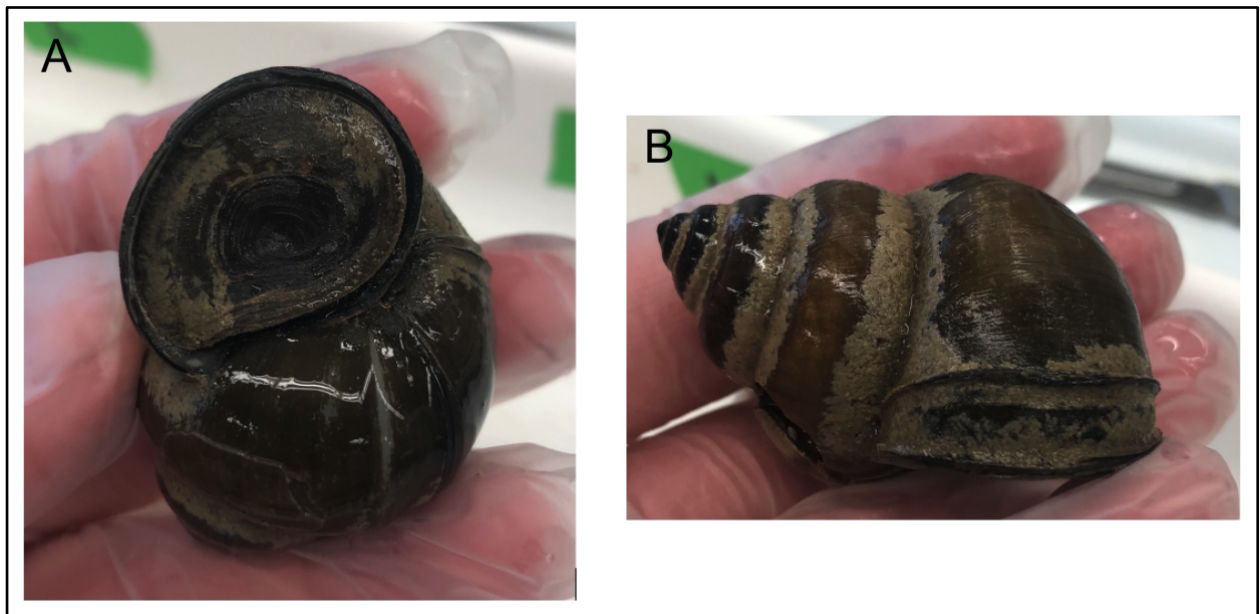


Figure 2.1. A) View of operculum on the ventral side of a Chinese Mystery Snail. B) Side view of a Chinese Mystery Snail. Images by Megan Edgar (University of Alberta).

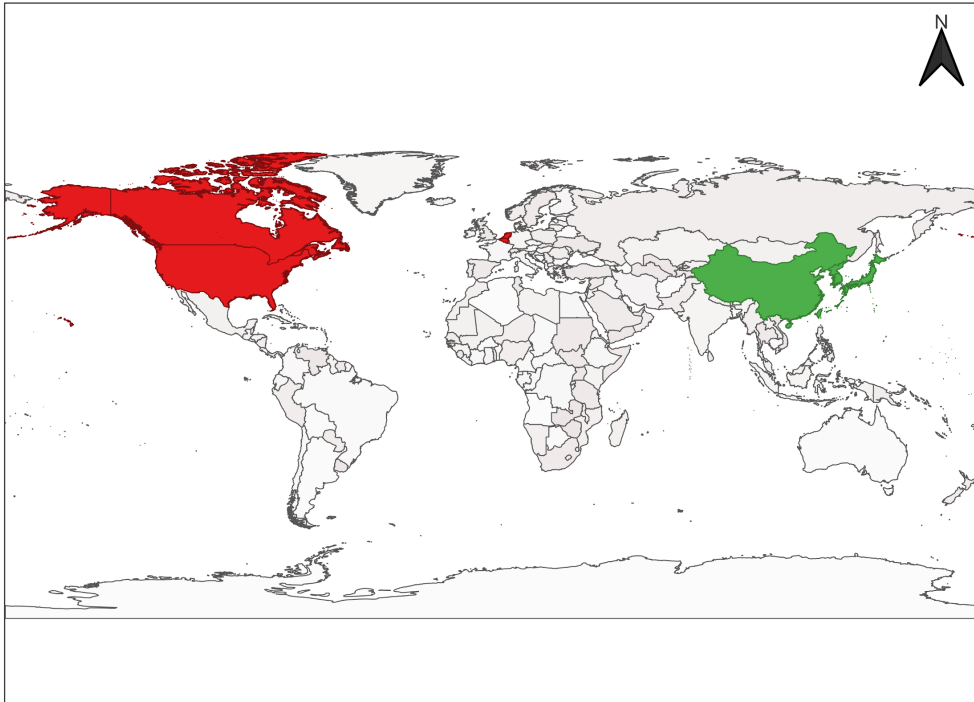


Figure 2.2. Map of countries with reported detections of Chinese Mystery Snail (*Cipangopaludina chinensis*). Countries in red represent invaded countries outside of the native range, whereas countries in green are designated to regions where Chinese Mystery Snails are native.

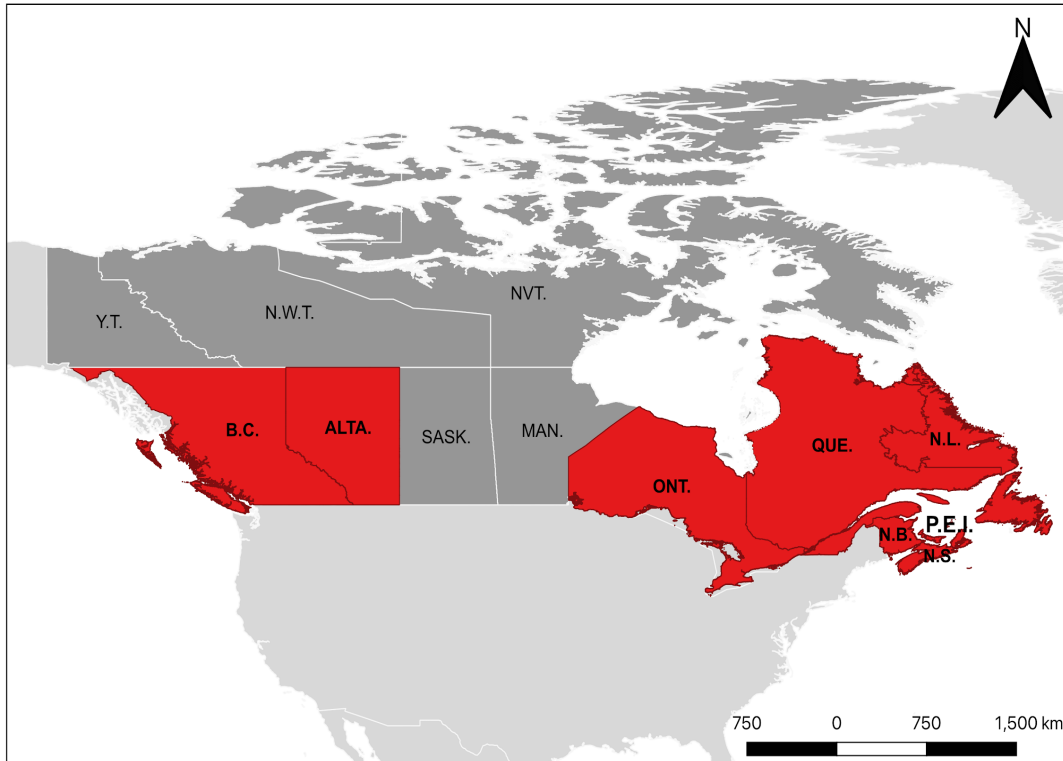


Figure 2.3. Map of provinces with reported detections of Chinese Mystery Snail (*Cipangopaludina chinensis*).

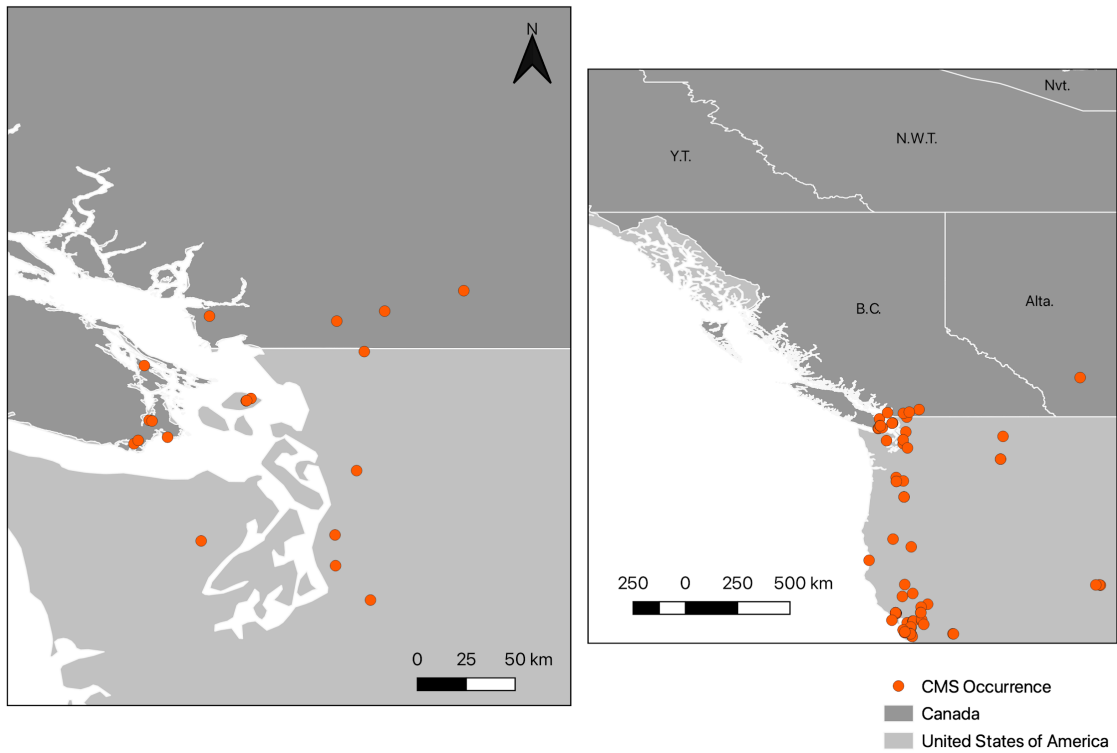


Figure 2.4. Occurrences of Chinese Mystery Snail (CMS) in western Canada and the United States of America. The map on the left is the extent of southern British Columbia (B.C.). Occurrence dataset points retrieved from GBIF (2021).

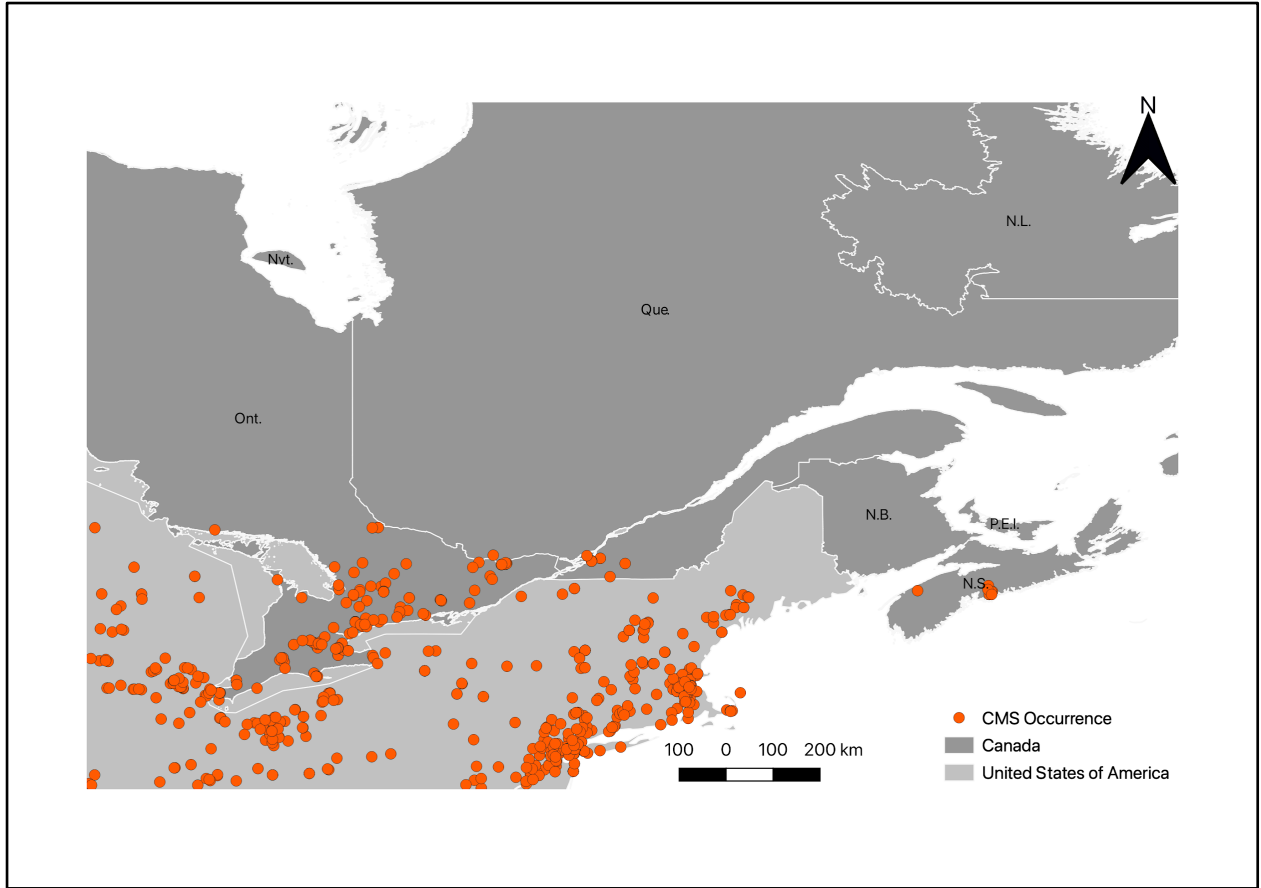


Figure 2.5. Occurrences of the Chinese Mystery Snail (CMS) in eastern Canada (Ontario, Quebec, New Brunswick, Nova Scotia), and the United States of America. Occurrence dataset points retrieved from GBIF (2021).

Chapter 3

The first documented occurrence and life history characteristics of the Chinese Mystery Snail, *Cipangopaludina chinensis* (Gray, 1834) (Mollusca: Viviparidae), in Alberta, Canada

Megan R. Edgar¹, Patrick C. Hanington², Robert Lu², Heather Proctor³, Ron Zurawell⁴, Nicole Kimmel⁴ and Mark S. Poesch^{1, *}

¹University of Alberta, Department of Renewable Resources, Edmonton, AB, Canada, T6G 2H1

²University of Alberta, School of Public Health, Edmonton, AB, Canada, T6G 1C9

³University of Alberta, Department of Biological Sciences, Edmonton, AB, Canada, T6G 2E9

⁴Alberta Environment and Parks, Edmonton, AB, Canada

* Corresponding author

Executive Summary

The Chinese Mystery Snail *Cipangopaludina chinensis* (Gray, 1834), a species native to Asia, is documented for the first time in Alberta, Canada, in McGregor Lake Reservoir in 2019. Here, we describe the initial finding of *C. chinensis* in Alberta, Canada, and biological information that may aid management efforts. Collected specimens were confirmed as *C. chinensis* through DNA barcoding. Analysis of growth rate, fecundity, and infection by digenean trematodes was assessed. It is unknown how *C. chinensis* arrived in Alberta. However, this species' ability to withstand environmental stressors, such as desiccation, facilitates overland and long-distance transport via recreationists or deliberate release of *C. chinensis* into waterbodies. Snails collected from McGregor Lake Reservoir matched with GenBank results for *C. chinensis* from Korea. Analysis of digenean trematodes revealed that the population in McGregor Lake are not infected, as there were no cercariae present after 24 hours. Growth assessment over a period of 60 weeks revealed that shell length growth quickly outpaces growth in shell width. Upon emergence, *C. chinensis* are larger than many native snail species. The expansion of *C. chinensis* into Alberta

poses potential negative consequences, such as decreased native snail biomass, increased nitrogen to phosphorus ratios, and additive impacts when paired with other invasive species.

Introduction

The Chinese Mystery Snail, *Cipangopaludina chinensis* is a viviparid mollusc native to Asia (Jokinen 1982; Solomon et al. 2010). *Cipangopaludina chinensis* has two subspecies, *C. C. chinensis* (Gray, 1834) and *C. c. laeta* (Martens, 1860). Here, we focus on *C. chinensis* clade introduced into North America through San Francisco, California, in the 1890s (Kingsbury et al. 2021). *C. chinensis* are well established in the United States, with the largest populations in the upper midwest and northeastern states (McAlpine et al. 2016; Kipp et al. 2014). In Canada, *C. chinensis* are established in southern Ontario, including Lake Erie, western Lake Ontario, the Kawartha Lakes, and Crowe, Moira and Trent River drainages (Therriault and Kott 2002), Québec (Clarke 1981; Tornimbeni et al. 2014), British Columbia (Clarke 1981), and Atlantic Canada (Nova Scotia, New Brunswick Prince Edward Island, Newfoundland (McAlpine et al. 2016; Kingsbury et al. 2021)).

Cipangopaludina chinensis were first reported in 2019 in Alberta, Canada, from McGregor Lake Reservoir, near the Village of Milo. Date of introduction, population history, and extent of dispersal from the first introduction at the site are unknown. Before discovery, *C. chinensis* were listed under the Fisheries Act of Alberta as a prohibited species, making it illegal to possess, release, sell or transport the species within the province (Alberta Environment and Parks 2019). Further, federal legislation under The Fisheries Act, Aquatic Invasive Species Regulations (Government of Canada 2015) prohibits the possession and sale of *C. chinensis* in Alberta. Despite these prohibitions, *C. chinensis* are sold as a food item in Asian food markets

and in the aquarium and pet trade across North America (Wyman-Grothem et al. 2018). Human dispersal of *C. chinensis* and seeding of water bodies are likely important in spreading this species in North America.

Research suggests that *C. chinensis* are challenging to eradicate once established. Difficulty in eradication is a direct consequence of the operculum, which protects the species internal viscera from desiccation (Figure 1). *C. chinensis* may survive out of water for up to nine weeks, and chemical means of population control, including traditional molluscicides such as rotenone and copper sulphate, are apparently ineffective (Haak et al. 2014; Unstad et al. 2013). According to Unstad et al. (2013), *C. chinensis* cannot be managed via culling methods or drawdowns. Burnett et al. (2018) found *C. chinensis* exhibits an upper thermal tolerance of 45 °C but they did not determine a lower lethal temperature limit, thus further supporting its probable overwintering ability in regions with colder winter temperatures like Alberta. Within their indigenous range *C. chinensis* serves as a host for several helminth parasites that affect humans, including human intestinal fluke (Chung and Jung 1999). Conceivably, parasites that may negatively impact non-native ecosystems can be introduced from *C. chinensis* (Harried et al. 2015).

This paper reports our initial research and monitoring efforts on the occurrence and implications of *C. chinensis* in Alberta. Monitoring and tracking the occurrence and spread of non-native *C. chinensis* in Canada is key to mitigating potential ecological and economic harm (Lodge and Shrader-Frechette 2003). Research and monitoring often focus on charismatic and game species (Thomsen et al. 2014; Tensen 2018) such as sportfish or the organisms that impact them (i.e. whirling disease; Mayhood 2000; Bartholomew et al. 2005). This may allow less

conspicuous organisms, such as benthic invertebrates, to spread unnoticed until they become problematic in an ecosystem.

Material and methods

Cipangopaludina chinensis were first observed by one of the authors (RL) in McGregor Lake Reservoir, Alberta, in 2019. McGregor Lake Reservoir (50.5696°N; 112.8828°W; WGS84) is an off-stream storage reservoir built in 1920 next to the Village of Milo and situated approximately 30 kilometers east of the town of Vulcan in southern Alberta, Canada (Mitchell et al. 1990) (Figure 3.1). McGregor Lake Reservoir is part of the Oldman River drainage basin, although most water input is via diversion from the Bow River (Mitchell et al. 1990). Immediately downstream to the south and then south-east of McGregor Lake Reservoir are Travers and Little Bow Lake Reservoirs, respectively (Figure 3.1). The three reservoirs are part of the Carseland-Bow River Headworks system. Travers and Little Bow Reservoirs do not currently have any reports of *C. chinensis*.

In October 2019 and August 2020, visual shoreline surveys were conducted. *C. chinensis* were surveyed for at nine different locations around McGregor Lake Reservoir in 2019, resulting in the collection of twenty-six live snails (Supplementary material Table S1; Figure 3.2). In 2020, searches at McGregor Lake, Travers Lake, and Little Bow Reservoir were surveyed more intensively to determine the extent of *C. chinensis* occurrence, utilizing an Eckman dredge and kick samples to collect benthic samples. An additional 58 live snails were collected. Species was first identified morphologically following a key created by Lu et al. (2014). Broad similarities to other viviparid species, notably the Japanese mystery snail, *C. japonica* (von Martens, 1861), prompted molecular investigation, the preferred method for distinguishing the species

(Kingsbury et al. 2021). The collected snails were further used to assess growth rate, fecundity, and digenean trematode infection.

DNA barcoding

Genomic DNA from tentacle clips taken from three individual snails was extracted using the DNeasy Blood and Tissue kit (Qiagen, USA). A ~ 710 fragment of cytochrome c oxidase 1 (CO1) was amplified using the forward and reverse primer pairs published by Folmer et al. (1994): (HCO2198 and LCO 1490). Endpoint Polymerase Chain Reaction (PCR) was carried out in a 50 µl reaction mixture using the following cycling parameters: 92 °C for 2 min, 92 °C for 40 s, 51 °C for 1 min, 68 °C for 1 min and 68 °C for 7 min. Amplicons were visualized on 1% agarose gels, and bands at the expected size were extracted and purified using the QIAquick Gel Extraction Kit (Qiagen, USA). Sanger sequencing using the HCO2198 and LCO1490 primers was carried out on the purified amplicons (Macrogen Inc, South Korea). Sequences were input into Geneious version 11.0.6 (Kearse et al. 2012; <http://www.geneious.com>), trimmed, aligned, and the resulting consensus sequences were queried against the BLASTn database (Zhang et al. 2000).

Phylogenetic analysis

All CO1 sequences were checked for quality by viewing chromatograms and quality scores in 4peaks (Nucleobytes) software. Primer regions were trimmed and transferred to Geneious Prime 2019 (<https://www.geneious.com>) to align the forward and reverse sequences. The three 659 base pair consensus sequences were identical to each other. The representative sequence

(GenBank # OK147091) was then compared using BLASTn against the NCBI GenBank database. GenBank submissions representing *C. chinensis*, or closely related species were selected for subsequent phylogenetic analysis. When possible, submissions for which voucher specimens had been submitted were used. Geneious Prime was used to create nucleotide alignments for the selected sequences. Alignments were trimmed to the shortest sequence length (which was the 530 nucleotide outgroup CO1 sequence) prior to analysis. Bayesian inference (BI) reconstructions were generated using the Mr. Bayes plug-in (Ronquist and Huelsenbeck 2003) in Geneious Prime with a burn-in of 100,000, a chain length of 1,000,000, and subsampling frequency of 200. Maximum-likelihood (ML) analyses were run in the PhyML plug-in (Guindon et al. 2010) for separate genus-level analysis. The settings used were: 200 bootstraps, proportion of invariable sites was fixed at 0, the number of substitution rate categories was 4, the gamma distribution parameter was set to estimated and “topology/length/rate” was selected to be optimized. GTR + invgamma was the best-supported nucleotide substitution model available in the MrBayes plug-in in Geneious for BI analyses. The CO1 sequence for *Viviparus contectus* isolate 1Z33 (MK517422.1) was used as an outgroup for the phylogenetic tree.

Assessment of fecundity and growth

Snails collected in 2019 were assessed for the presence of offspring. Those *C. chinensis* with offspring (n = 6) were separated into individual aquaria in the lab and furnished with 10 cm of autoclaved substrate collected from Lake McGregor. Snails without offspring were dissected and sexed based on structures describe by Simone (2011) (16 males; 4 females). After offspring emerged, the adult female snails were euthanized and dissected to confirm that all offspring had emerged. Juvenile snails were kept in the same aquaria and individually marked using coloured enamel paint before measuring shell width and length. Juvenile snails were fed algae pellets

(Hikari, USA) weekly and kept at 17 °C in a room with a 12-hour day/night cycle. Shell morphometrics were collected weekly until all snails had died (60 weeks).

Assessment for infection by digenean trematodes

The 84 snails from 2019 and 2020 were isolated individually in containers with artificial spring water at a depth of ~ 3 cm for 24 hours in a 12 hour day-night cycle (Gordy and Hanington 2019), the water was then assessed to see if any trematode cercariae had emerged from the snails, using a Zeiss V16 stereomicroscope.

Results

Visual shoreline surveys in October 2019 confirmed the presence of *C. chinensis* at McGregor Lake at the north end of the Reservoir (Table S1). *Cipangopaludina chinensis* shells found on beaches south of the boat launch ranged from 5 to 50 mm in length. No *C. chinensis* were found in Travers Lake Reservoir (Figure 3.1; Table S3.2).

All three snails for which CO1 sequences were obtained returned a 100% nucleotide identity to GenBank submission # MN997925.1, identified as a *Cipangopaludina chinensis* specimen (voucher ZMB 192694) from Korea. The Alberta *C. chinensis* (OK147091) formed a monophyletic group with other *C. chinensis* (Figure 3.3), confirming the results of the BLASTn analysis.

Six female *C. chinensis* yielded offspring that were assessed for growth over 60 weeks. A total of 116 juvenile snails emerged from the females. The mean number of juveniles was 19.3, with 25, 23, 23, 19, 13 and 13 individual juveniles emerging from each female (Table S3.2).

Upon emergence, mean shell width was 5.13 ± 1.45 mm and mean shell length was 5.9 ± 1.6 mm. The width:length ratio on the day of emergence was 0.87 ± 0.02 ($n = 116$). Weekly measurement of shell width and length over a 60-week period indicates that shell length growth (slope of 0.2821) outpaces growth in shell width (slope of 0.209) with a width:length ratio of 0.76 ($n = 1$) measured on week 60 (Figure 3.4).

None of the 84 snails assessed for digenean trematode infection produced any cercariae after 24 hours.

Discussion

We confirm the presence of non-native *C. chinensis* in Alberta, Canada, notably at the north end of McGregor Lake Reservoir, where the boat launch recreation areas are located (Figure 3.2). We suggest that at McGregor Lake Reservoir *C. chinensis* has met criteria for four of the five stages of species invasion: arrival, establishment, growth, and reproduction (Lockwood et al. 2007). As of yet there is no evidence to confirm the fifth stage, dispersal, of *C. chinensis* from McGregor Lake Reservoir to other waterbodies in Alberta. The two closest waterbodies, Little Bow and Travers Lake Reservoirs, have been surveyed but to date found to be negative for *C. chinensis*. Should *C. chinensis* disperse to these adjacent water bodies, continuous monitoring at these sites will be required for early detection.

DNA barcoding of CO1 suggests that the snails invading McGregor Lake Reservoir are *C. chinensis*. This conclusion is supported by phylogenetic analysis that groups the Alberta CO1 sequences, which are identical to each other, in a monophyletic group with other *C. chinensis*. Our phylogenetic analysis closely resembles recently constructed phylogenies for *C. chinensis* (David and Cote 2019).

Our study determines that populations of *C. chinensis* in Alberta grow allometrically; shell width to shell height ratio decreases as the shell increases in size, from juvenile to adult life stages, as also reported from studies by Jokinen (1982). Growth assessment of *C. chinensis* affirms that juvenile *C. chinensis* emerge larger than some adult native species in Alberta (Clifford 1991), and that adult *C. chinensis* are larger than any native freshwater gastropod species (Liu et al. 1995; Kingsbury 2021). The large size of *C. chinensis*, when compared to native snails, may facilitate their avoidance of predators in Alberta waterbodies. A study by Johnson et al. (2009) saw that when non-native *C. chinensis* co-occurs with native snails, crayfish attack the native snails at a higher rate than *C. chinensis*, as a result of their larger size and thicker shell, exacerbating predation on native gastropods. Already, *C. chinensis* is documented to have facilitative interactions with invasive species in their introduced regions (Haak 2015). This suggests that *C. chinensis* may interact with Northern Crayfish (*Faxonius virilis*), a crayfish that has recently expanded its native range from the Beaver River drainage of Alberta (Williams et al. 2011). Further, a behavioural study by Sura and Mahon (2011) determined that the presence of crayfish, as detected by chemical cues, causes native snails to decrease their feeding rates relative to the absence of crayfish; a costly behavioural change, as this effects metabolism, growth, and reproduction. A report from 2013 determined that 74% of freshwater gastropods are presently suffering declines (Johnson et al. 2013). Where *C. chinensis* is introduced, there is a potential that native snails will have much lower feeding rates, especially where crayfish are present (Sura and Mahon 2011), presenting a facilitative interaction between two non-natives (Ricciardi 2001).

Annual fecundity on average is between 25 and 30 juveniles per female (Stephen et al. 2013), with specimens from Alberta producing offspring numbers somewhat below this at 19.3 juveniles per female. However, previous research has shown that even one gravid female is sufficient to found a population, with *C. chinensis* producing young every year until death (Stephen et al. 2013). Female *C. chinensis* also grow larger than male conspecifics (Jokinen 1982), and because of predator aversion due to their larger size, and these characteristics could facilitate reproduction and survival in Alberta, and potential spread.

We did not detect any *C. chinensis* from McGregor Lake infected with larval digenean trematodes. Parasites detected in *C. chinensis* in North America include *Cyathocotyle bushiensis*, a parasite known to cause mortality in dabbling ducks (Hoeve and Scott 1988), and *Aspindogaster conchicola*, a parasite known to infect fish, freshwater bivalves and turtles (Harried et al. 2015; Kingsbury 2021). According to Karatayez et al. (2012), parasite prevalence in *C. chinensis* is much lower than in native snail species. However, continual monitoring for parasites on *C. chinensis* is needed as warmer water temperature could result in an increase in the prevalence of parasites. Previous research has shown that metacercarial infections were found in snails in Lake Wabuman, Alberta, where water was kept warm year-round due to thermal effluents, at temperatures between 5 and 24 °C (Sankurathri and Holmes 1976).

Based on our findings, survey efforts focussed on water depths of 1–4 meters may be the most effective for detecting snail presence (Table S1). Extra consideration should be focussed on areas with artificial substrates, riprap, submerged vegetation (Chaine 2012), and where there are stagnant waters (Perron and Probert 1973). Vigilance should be taken near the northern end of McGregor Lake Reservoir, where the boat launch and recreation area are located, as those location types are speculated to be critical factors for habitat suitability (Solomon et al. 2010). In

addition to potential impacts on native snails species, other documented and potential impacts of introduced *C. chinensis* in invaded water bodies include: increasing nitrogen to phosphorus ratios of surface water (Johnson et al. 2009); additive impacts to ecosystems when paired with other invasive species (Johnson et al. 2009; Olden et al. 2009); and potential food web effects (Sura and Mahon 2011; Kingsbury et al. 2020). Without management efforts that involve enforcing legislation now in place, public education, and continued monitoring, the range of the *C. chinensis* in Canada is only likely to expand.

Conclusions

Continued monitoring to track dispersal of *C. chinensis* in Alberta, Canada, is recommended. Further studies to document *C. chinensis* impact on aquatic systems in Canada and facilitate invasive species management are required. In Alberta, watershed managers should monitor Travers and Little Bow Lake Reservoirs adjacent to the McGregor Lake Reservoir, where *C. chinensis* is now established.

Acknowledgements

We would like to acknowledge Jacqueline Pallard and Matthew Cunningham from the Poesch Lab (Fisheries and Aquatic Conservation Lab), Mel Kuerht, Brendan Knowles and Imogen Grant-Smith from Alberta Environment and Parks (AEP), along with Michael Merrills and Dane Pearson from the Alberta Environmental Support and Emergency Response Team (ASERT) for their contributions surveying for the Chinese Mystery Snail. The authors extend sincere gratitude to anonymous reviewers and editors for their valuable comments on our manuscript.

Literature Referenced

- Alberta Environment and Parks (2019) Chinese Mystery Snail (*Cipangopaludina chinensis*) - Open Government. <https://open.alberta.ca/publications/chinese-mystery-snail-alert#detailed> (accessed 1 January 2021)
- Bartholomew JL, Kerans BL, Hedrick RP, Madiarmid, SC, Winton, JR (2005) A Risk Assessment Based Approach for the Management of Whirling Disease. Reviews in Fisheries Science 13: 205–230. <https://doi.org/10.1080/10641260500326842>
- Burnett JL, Pope K L, Wong A, Allen CR, Haak DM, Stephen BJ, Uden DR (2018) Thermal Tolerance Limits of the Chinese Mystery Snail (*Bellamya chinensis*): Implications for Management. American Malacological Bulletin 36:140–144. <https://doi.org/10.4003/006.036.0106>
- Chaine N (2012) Population estimate of Chinese Mystery Snail (*Bellamya chinensis*) in a Nebraska reservoir. BioInvasions Records 1: 283–287. <https://doi.org/10.3391/bir.2012.1.4.07>
- Chung PR, Jung Y (1999) *Cipangopaludina chinensis* malleata (Gastropoda: Viviparidae): A New Second Molluscan Intermediate Host of a Human Intestinal Fluke Echinostoma cinetorchis (Trematoda: Echinostomatidae) in Korea. The Journal of Parasitology 85: 963–964. <https://doi.org/10.2307/3285837>
- Clarke AH (1981) The freshwater molluscs of Canada. Ottawa: National Museum of Natural Sciences, National Museums of Canada, 456pp
- Clifford HF (1991) Aquatic Invertebrates of Alberta. University of Alberta Press, Edmonton, Alberta, 550 pp

- David A, Cote S (2019) Genetic evidence confirms the presence of the Japanese mystery snail, *Cipangopaludina japonica* (von Martens, 1861) (Caenogastropoda: Viviparidae) in northern New York. *BioInvasions Records* 8: 793–803.
<https://doi.org/10.3391/bir.2019.8.4.07>
- Folmer O, Black M, Wr H, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology* 3:294–9
- Gordy MA, Hanington PC (2019) A fine-scale phylogenetic assessment of digenean trematodes in central Alberta reveals we have yet to uncover their total diversity. *Ecology and Evolution* 9: 3153–3238. <https://doi.org/10.1002/ece3.4939>
- Government of Canada (2015) Fisheries Act, Aquatic Invasive Species Regulations. <https://laws-lois.justice.gc.ca/eng/regulations/sor-2015-121/FullText.html> (accessed 10 April 2021)
- Guindon S, Dufayard J-F, Lefort V, Anisimova M, Wim, Hordijk, Gascuel O (2010) New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Systematic Biology* 59:307–321.
<https://doi.org/10.1093/sysbio/syq010>
- Haak DM (2015) Bioenergetics and Habitat Suitability Models for The Chinese Mystery Snail (*Bellamya chinensis*). Ph.D. Thesis, University of Nebraska-Lincoln, 107 pp
- Haak DM, Stephen BJ, Kill RA, Smeenk NA, Allen CR, Pope KL (2014) Toxicity of copper sulfate and rotenone to Chinese Mystery Snail (*Bellamya chinensis*). *Management of Biological Invasions* 5: 371-375. <https://doi.org/10.3391/mbi.2014.5.4.08>
- Harried B, Fischer K, Perez KE, Sandland GJ (2015) Assessing Infection Patterns in Chinese Mystery Snails from Wisconsin USA Using Field And Laboratory Approaches. *Aquatic Invasions* 10: 169-175. <https://doi.org/10.3391/ai.2015.10.2.05>

- Hoeve J, Scott ME (1988) Ecological Studies on *Cyathocotyle bushiensis* (Digenea) and *Sphaeridiotrema globulus* (Digenea), Possible Pathogens of Dabbling Ducks In Southern Québec. *Journal of Wildlife Diseases* 24: 407–421. <https://doi.org/10.7589/0090-3558-24.3.40>
- Johnson PD, Bogan AE, Brown KM, Burkhead NM, Cordeiro JR, Garner JT, Hartfield PD, Lepitzki, DAW, Mackie GL, Pip E, Tarpley TA, Tiemann JS, Whelan NV, Strong EE (2013) Conservation Status of Freshwater Gastropods of Canada and the United States. *Fisheries* 38: 247–282. <https://doi.org/10.1080/03632415.2013.785396>
- Johnson PTJ, Olden JD, Solomon CT, Vander Zanden MJ (2009) Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia* 159:161–170. <https://doi.org/10.1007/s00442-008-1176-x>
- Jokinen E.J. 1982. *Cipangopaludina chinensis* (Gastropoda: Viviparidae) in North America, Review and Update. *Nautilus*, 96(3): 89–95.
- Karatayev AY, Mastitsky SE, Burlakova LE, et al (2012) Exotic Molluscs in the Great Lakes Host Epizootically Important Trematodes. *Journal of Shellfish Research* 31:885–894. <https://doi.org/10.2983/035.031.0337>
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649
- Kingsbury SE, McAlpine DF, Cheng Y, Parker E, Campbell, LM (2021) A review of the non-indigenous Chinese Mystery Snail, *Cipangopaludina chinensis* (Viviparidae), in North America, with emphasis on occurrence in Canada and the potential impact on indigenous

- aquatic species. *Environmental Reviews* 29(2): 182-200, <https://doi.org/10.1139/er-2020-0064>
- Kingsbury S, Fong M, McAlpine DF, Campbell L (2020) Assessing the probable distribution of the potentially invasive Chinese Mystery Snail, *Cipangopaludina chinensis*, in Nova Scotia using a random forest model approach. *Aquatic Invasions* 16(1): 167–185
- Kipp RM, Benson AJ, Larson J, Fusaro A (2014) *Cipangopaludina chinensis malleata*. USGS Nonindigenous Aquatic Species Database, Gainesville, FL and NOAA Great Lakes Aquatic Nonindigenous Species Information System, Ann Arbor, MI, [https://nas.er.usgs.gov/queries/greatlakes/FactSheet.aspx?Species_ID=1045&Potential=N&](https://nas.er.usgs.gov/queries/greatlakes/FactSheet.aspx?Species_ID=1045&Potential=N&RevisionDate=11/6/2019), Revision Date: 11/6/2019 (accessed 11 March 2022)
- Liu YY, Zhang WZ, Wang YX (1995) Distribution of the family Viviparidae from China (Mollusca: Gastropoda). In: *Transactions of the Chinese Society of Malacology*, n.p., pp 5-6.
- Lockwood JL, Hoopes MF, Marchetti MP (2007) *Invasion Ecology*, Second Edition. Wiley-Blackwell, Malden Massachusetts, 456pp
- Lodge DM, Shrader-Frechette K (2003) Nonindigenous Species: Ecological explanation, environmental ethics, and public policy. *Conservation Biology* 17: 31-37
- Lu H-F, Du L-N, Li Z-Q, Chen X-Y, Yang J-X (2014) Morphological analysis of the Chinese *Cipangopaludina* species (Gastropoda; Caenogastropoda: Viviparidae). *Zoological Research* 35: 18
- Mayhood DW (2000) Provisional Evaluation of the Status of Westslope Cutthroat Trout in Canada. In: Darling LM (eds) (2000) *Proceedings of a Conference on the Biology and Management of Species and Habitats at Risky, Kamloops, BC, February 15-19, 1999*. 2: 579-585

- McAlpine D, Lepitzki D, Schueler F, McAlpine A, Hebda A, Forsythe R, Nicolai A, Maunder J, Noseworthy R (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: 149–154. <https://doi.org/10.3391/bir.2016.5.3.05>
- Mitchell P, Prepas EE, Crosby JM (1990) *Atlas of Alberta Lakes*. University of Alberta Press: 675pp
- Olden J, Larson E, Mims M (2009) Home-field advantage: Native signal crayfish (*Pacifastacus leniusculus*) out consume newly introduced crayfishes for invasive Chinese Mystery Snail (*Bellamya chinensis*). *Aquatic Ecology* 43: 1073-1084. <https://doi.org/10.1007/s10452-009-9244-9>
- Perron F, Probert T (1973) *Viviparus malleatus*, new record in New Hampshire. *Nautilus* 87: 90
- Ricciardi A (2001) Facilitative interactions among aquatic invaders: is an “invasional meltdown” occurring in the Great Lakes? *Canadian Journal of Fisheries and Aquatic Science* 58: 2513-2525. <https://doi.org/10.1139/cjfas-58-12-2513>
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Sankurathri CS, Holmes JC (1976) Effects of thermal effluents on parasites and commensals of *Physa gyrina* Say (Mollusca: Gastropoda) and their interactions at Lake Wabamun, Alberta. *Canadian Journal of Zoology* 54: 1742–1753. <https://doi.org/10.1139/z76-202>
- Simone L (2011) Phylogeny of the Caenogastropoda (Mollusca), based on comparative morphology. *Arquivos de Zoologica* 42(2-4): 83-323
- Solomon CT, Olden JD, Johnson PTJ, Dillon RT, Zanden MJV (2010) Distribution and community-level effects of the Chinese Mystery Snail (*Bellamya chinensis*) in northern

Wisconsin lakes. *Biological Invasions* 12: 1591–1605. <https://doi.org/10.1007/s10530-009-9572-7>

Stephen BJ, Allen CR, Chaine NM, Fricke KA, Haak DM, Hellman, ML, Kill RA, Nemec KT, Pope, KL, Smeenk NA, Uden DR, Unstad KM, VanderHam AE, Wong A (2013) Fecundity of the Chinese Mystery Snail in a Nebraska reservoir. *Journal of Freshwater Ecology* 28: 439–444. <https://doi.org/10.1080/02705060.2013.769127>

Sura SA, Mahon HK (2011) Effects of Competition and Predation on the Feeding Rate of the Freshwater Snail, *Helisoma trivolvis*. *The American Midland Naturalist* 166: 358–368

Tensen L (2018) Biases in wildlife and conservation research, using felids and canids as a case study. *Global Ecology and Conservation* 15: e00423
<https://doi.org/10.1016/j.gecco.2018.e00423>

Therriault T, Kott E (2002) *Cipangopaludina chinensis malleata* (Gastropoda: Viviparidae) in southern Ontario: An update of the distribution and some aspects of life history. *Malacological Review* 36: 111–121

Thomsen MS, Wernberg T, Olden, JD, Byers JE, Bruno JF, Silliman BR, Schiel DR (2014) Forty years of experiments on aquatic invasive species: are study biases limiting our understanding of impacts? *NeoBiota* 22:1-22

Tornimbeni O, Galvez R, Triffault-Bouchet G, Dassylva N, Roberge S (2014) Heavy metal concentrations in *Cipangopaludina chinensis* (Reeve, 1863) and relationships with sediments in Saint-Augustin Lake, Québec City (Quebec, Canada). *Annual Limnology - International Journal of Limnology* 49: 21–29, <https://doi.org/10.1051/limn/2013034>

Unstad K, Uden D, Allen C, Chaine D, Haak D, Kill R, Pope K, Stephen B, 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: 123–127, <https://doi.org/10.3391/mbi.2013.4.2.04>

Williams BW, Proctor HC, Clayton T (2011) Range Extension of The Northern Crayfish, *Orconectes virilis* (Decapoda, Cambaridae), In the Western Prairie Provinces of Canada. *Crustaceana* 84: 451-460

Wyman-Grothem KE, Himes H, Jewell S, Savignano D, Maclean D (2018) Chinese Mystery Snail (*Cipangopaludina chinensis*) ecological risk screening summary. U.S. Fish and Wildlife Service. <https://www.fws.gov/sites/default/files/documents/Ecological-Risk-Screening-Summary-Chinese-Mystery-Snail.pdf> (accessed 11 March 2022)

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7(1-2): 203-214

Figures

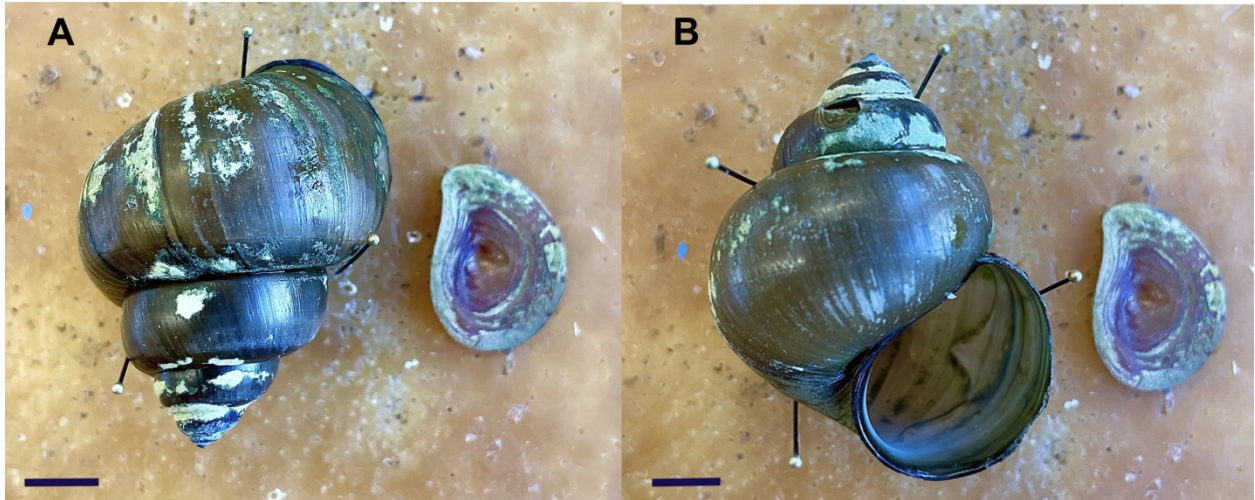


Figure 3.1. Shell of an individual *C. chinensis* from McGregor Lake Reservoir, Alberta, Canada. A. Abapertural view. B. Apertural view. Scale bar = 1 cm. Photo by: Megan R. Edgar.

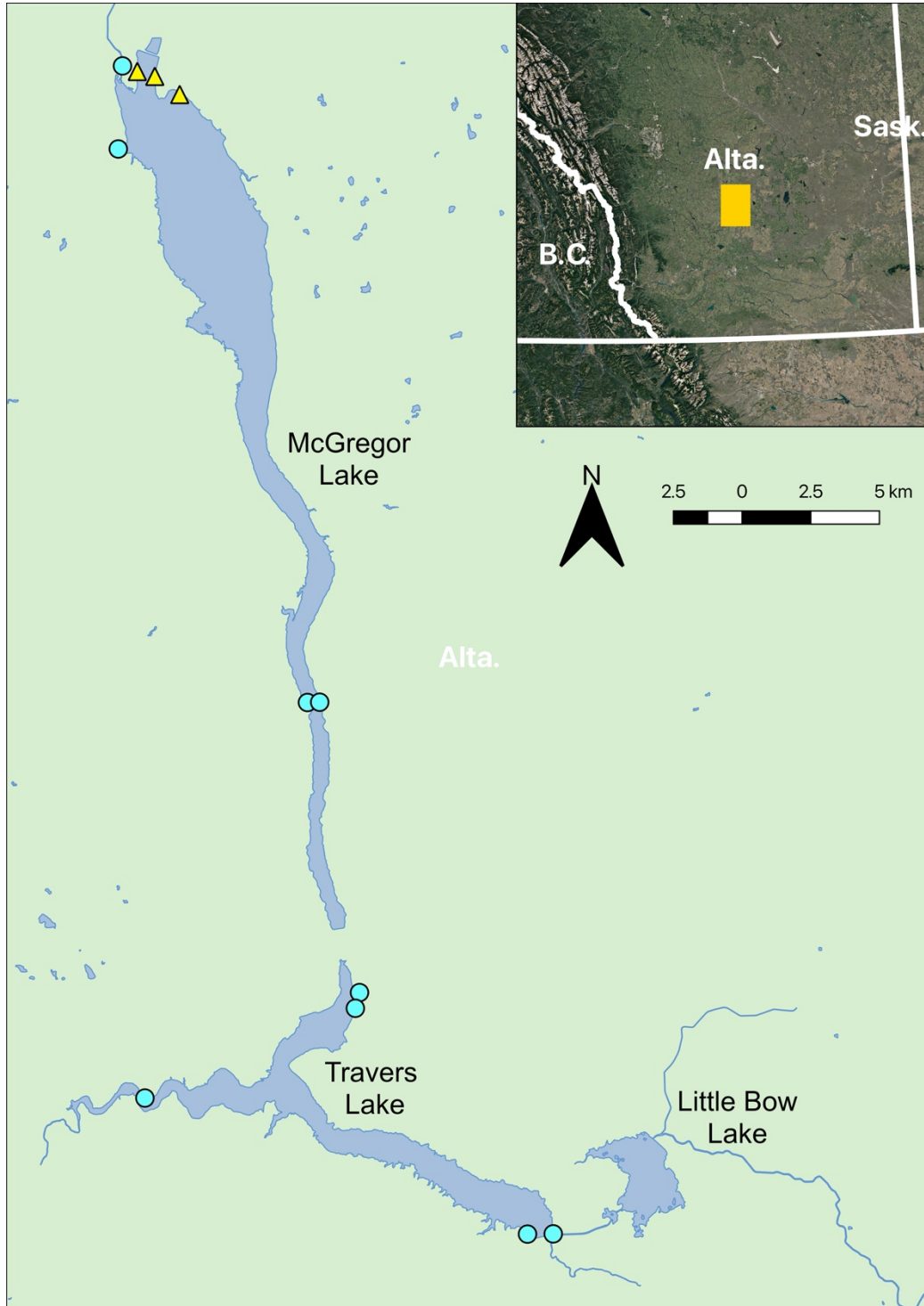


Figure 3.2. Location of sites surveyed for Chinese Mystery Snail in McGregor Lake and Travers Lake, Alberta, Canada. The inset map shows the survey area relative to British Columbia (B.C.), Saskatchewan (Sask.) and the United States of America boundary. Yellow triangles = *C. chinensis* detected; blue circles = *C. chinensis* not detected.

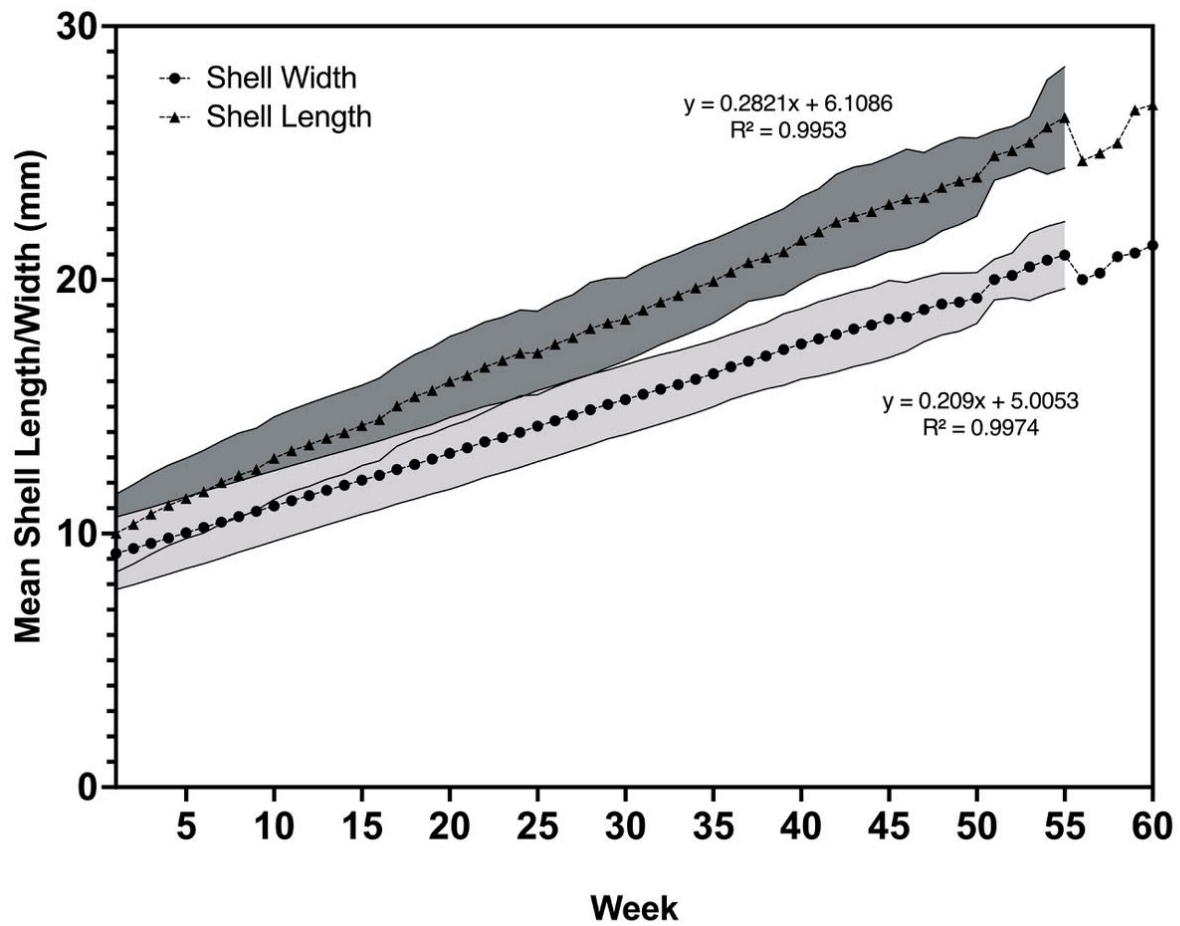


Figure 3.4. The growth of juvenile *Cipangopaludina chinensis* from McGregor Lake Reservoir, Alberta, Canada. Shell length (dark grey) rapidly outpaces an increase in shell width (light grey) over the first 60 weeks of life. Starting population size of this study was 116 juvenile snails originating from six females. No snails survived to week 60. The shaded areas represent the standard deviation.

Supplementary Materials

The following supplemental material is available for this article.

Table S3.1: Visual shoreline surveys for *C. chinensis* at seven locations on McGregor Lake (Figure 1) and two at Travers Reservoir, conducted by AEP and ASERT in October 2019. Note: Little Bow Reservoir and Little Bow Provincial Park are different. Little Bow Provincial Park is on Travers Reservoir, whereas Little Bow Reservoir is its own waterbody.

Reservoir	Site	Presence of <i>C. chinensis</i>	Comments
McGregor	Supply canal to McGregor Lake	Negative	-
McGregor	Parks Recreational Area (west shore)	Positive	A high number of shells
McGregor	East shore (opposite to Recreational Area)	Positive	A high number of shells
McGregor	Wetland near Highway 842 (south of Recreational Area)	Negative	-
McGregor	Shoreline southwest of the town of Milo	Positive	Shells present, though significantly less than the Parks Recreational Area and the East shore
McGregor	Highway 531 bridge over McGregor Lake (north shore)	Negative	-
McGregor	Highway 529 bridge, over McGregor Lake (south canal)	Negative	-
Travers	Little Bow Provincial Park (boat launch and beach)	Negative	-
Travers	Travers Reservoir (south shorelines along Township Road 142)	Negative	-

Table S3.2: Measurement data collected during dissections of *C. chinensis*, collected in 2020. Samples were collected from McGregor Lake, Alberta.

Site	Collection Date	Sampling method	Depth collected (m)	Length (mm)	Shell height (mm)	Weight (g)	Count of juveniles	Total wet weight of juveniles (g)
McGregor Lake	17-08-2020	Incidental	na	41	29.1	21.69	20	0.96
McGregor Lake	17-08-2020	Eckman dredge	4	43.9	25.3	15.35	22	1.52
McGregor Lake	17-08-2020	Eckman dredge	3.4	35	33	10.88	18	0.78
McGregor Lake	12-10-2019	Shoreline sampling	~1m	51.3	37.5			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	45	31.7		25	
McGregor Lake	12-10-2019	Shoreline sampling	~1m	30.8	24.6			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	19.2	17.9			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	31.9	26.4		23	
McGregor Lake	12-10-2019	Shoreline sampling	~1m	40.9	29.7			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	27.5	21.6			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	42.2	30.5		23	
McGregor Lake	12-10-2019	Shoreline sampling	~1m	24.6	19.8		13	
McGregor Lake	12-10-2019	Shoreline sampling	~1m	27.3	22.7			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	30.8	25		19	
McGregor Lake	12-10-2019	Shoreline sampling	~1m	42.8	31.8			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	19.2	16.4			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	29.2	24.2		13	
McGregor Lake	12-10-2019	Shoreline sampling	~1m	37	26.7			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	31	24.3			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	38.8	29.6			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	39.3	30.7			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	35.1	27.3			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	29.9	24.9			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	43.7	33			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	39.9	30.2			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	39.7	29.5			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	34	26.8			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	38.2	28.6			
McGregor Lake	12-10-2019	Shoreline sampling	~1m	26.1	21			

Chapter 4

Group and community trophic structure within reservoirs in Southern Alberta in the presence of *Cipangopaludina chinensis* and *Faxonius virilis*

Executive Summary

We conducted a study using multiple dietary metrics ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and gut-content analysis) to investigate diet shifts in reservoir systems where there are two sympatric aquatic invasive species (*Cipangopaludina chinensis* and *Faxonius virilis*), where there is one invasive species (*F. virilis*), and where there are none. We assessed reservoir populations of three main recreational fish species (Northern Pike (*Esox lucius*), Lake Whitefish (*Coregonus clupeaformis*), Walleye (*Sander vitreus*)) to compare resource use in each lake and to investigate whether invasive species may be altering this. We applied a Bayesian mixing model and determined that macrophyte proportion in diets of native recreational fish species changes with the addition of only one aquatic invader in the system. *C. chinensis* and *F. virilis* are competing for niche space, which may be limiting the adverse impact caused by the addition of another invasive species into that aquatic ecosystem. All three recreational species are experiencing diet switching to a more piscivorous diet when sharing an ecosystem with aquatic invaders, which may be due to reduced energy content of the diet when *C. chinensis* and *F. virilis* are added to the ecosystem.

1. Introduction

Invasive species are among the leading causes of biodiversity decline globally. As non-native species become established, they can out-compete or predate on native species, changing food web structure and altering ecosystem function (Olden et al. 2009; Drake and Mandrak 2014). Additionally, non-native species can alter trophic dynamics, including the fundamental flows of nutrients and energy (Schindler and Scheuerell 2002). Invasive species are spread through multiple different modes, including intentional stocking, aquarium releases, canal construction, international shipping (Rahel and Olden 2008), the ornamental pet trade, food markets, and recreationists (Jokinen 1992). Climate change and non-native species may react synergistically (Rahel and Olden 2008), which compounds the need to understand the complex nature of interactions in food webs where non-native species have been introduced.

Chinese Mystery Snail (*Cipangopaludina* [=*Bellamya*] *chinensis* Gray, 1834) and Northern Crayfish (*Faxonius virilis*), are two invasive species found in Alberta, Canada. *C. chinensis* are native to Asia, specifically to China, Taiwan, Korea, Japan, and Russia (Kingsbury et al. 2020). They arrived in North America in the late 1890s and it is speculated that they were introduced through food markets in San Francisco and radiated outward from that region. Since then, *C. chinensis* have established in 34 of the United States, and in Canada they are found in Newfoundland and Labrador, Ontario, Nova Scotia, New Brunswick, British Columbia, and Alberta (Kingsbury et al. 2020). *C. chinensis* is a relatively recently introduced species in Alberta (Edgar et al. 2022). In 2019, this species was officially documented to have established in McGregor Lake Reservoir. Due to their recent and localized arrival in Alberta, there is little known about how they may impact aquatic ecosystems. They are known to have adverse effects on natural ecosystems outside of their native range. Studies involving *C. chinensis* in other

regions have shown that they can reduce algal species composition and biomass, increase nitrogen to phosphorous ratios, promote eutrophication, and potentially alter food webs (Kingsbury et al. 2021; Haak 2011.).

Canada has 11 species of crayfish, with *F. virilis* being the most widespread species. This species is historically known to naturally occur east of the Continental Divide, from Saskatchewan to Québec (Phillips et al. 2009; McAlpine et al. 1999; Clark and Lester, 2005), but have expanded their range from Saskatchewan to Alberta (Van Mierlo 2022). They can now be found throughout Alberta, including the North and South Saskatchewan River basins. *F. virilis* has the potential to affect the benthic ecosystems and have adverse consequences for waterbodies in invaded regions, if left unchecked (Phillips et al. 2009, Van Mierlo 2022). In many ecosystems, crayfish species are often the largest invertebrate present (Momot 1984). Studies have noted that their ability for aggressive predation and habitat alteration can make them particularly impactful in regions that previously lacked these species (Momot 1984; Phillips et al. 2009). Previous work in the United States Midwest shows that lakes that were invaded by crayfish altered the feeding behaviour of predatory fishes, pushing them to feed in the littoral zone and leading them to occupy a lower trophic position (Nilsson et al. 2012; Prestie et al. 2019; Kreps et al., 2016).

There have been studies investigating the interactions between *C. chinensis* and various crayfish species in lab and mesocosm settings, but fewer looking at these interactions in natural, large-scale settings. Previous work shows that Rusty crayfish (*Orconectes rusticus*), a species physiologically similar to *F. virilis*, are able to consume *C. chinensis*. This suggests that the two species may interact in locations where they have both established (Solomon et al. 2010).

Another study concluded that the dual effects of predation by crayfish and competition with *C. chinensis* was found to have additive impacts on native snails than if there were only one non-native species (Johnson et al. 2009). In Washington, USA, *C. chinensis* may have exacerbated the abundance of *F. virilis*, by providing an abundant prey source (Olden et al. 2013).

Food web structure is a measure of energy flow within an ecosystem. Food webs can be identified by using stable isotopes of carbon and nitrogen. These stable isotopes are a powerful method for estimating trophic positions and carbon flow to consumers in food webs. Stable isotope analysis and Bayesian mixing models are two of the most important tools for examining the structure and dynamics of food webs (Saporiti et al. 2015). Carbon isotopes describe the primary energy source and can show the difference between benthic and pelagic primary producers. Stable isotopes of nitrogen show the boundaries between trophic levels, therefore helping determine the trophic position of a species (O'Reilly et al. 2002). Invasive species are known to potentially change food web structures, by lengthening or shortening food chain length, and impacting the remaining organisms in an ecosystem (Prestie et al. 2019; Cabana and Rasmussen, 1994; Vander Zanden and Rasmussen 1996). Moreover, invasive species often had wider niches and a higher abundance and plasticity in resource use compared to native species, increasing their competitive capabilities (Tilman 1999; Yalçın Özdilek et al. 2019).

Our study investigates the impacts of both *C. chinensis* and *F. virilis* on a large scale. The objectives of this study were to 1) determine the difference in trophic characteristics between reservoir communities where invasive species are sympatric, where only invasive crayfish are present, and where there are no invasive species present, and; 2) describe the trophic interaction between *C. chinensis* and *F. virilis* in aquatic food webs. I hypothesize that with the presence of

only one aquatic invader will create bottom-up effects on the food-web, and an additional invasive species will amplify these effects, creating shifts in fish trophic positions by moving basal energy away from the higher trophic levels through consumption of algae and macrophytes, or by providing a new prey resource (Twardochleb and Olden 2016; Kingsbury et al. 2021). I also hypothesize that *C. chinensis* and *F. virilis* will have overlapping niches, either through competition for resources or through the predation of *C. chinensis* by *F. virilis* (Johnson et al. 2009). We used stable isotope analysis and gut-content analysis, jointly, to help determine answers and address these objectives.

2. Methods

2.1. Field sampling

Field sampling occurred at five lake reservoirs in southern Alberta in August and September of 2020 and 2021. There were three treatments: (1) reservoirs that have *C. chinensis* and *F. virilis*, (2) reservoirs where there are only *F. virilis*, and (3) the reference treatment where there were no invasive species found or reported. McGregor Lake Reservoir is occupied by both invasive species, Travers Lake, Sherburne Lake, and 40-Mile Coulee Reservoir were occupied by *F. virilis* only, and St. Mary's Reservoir acted as a reference system (i.e., neither invasive species present). There were no reservoirs with only *C. chinensis* as it occurs only in McGregor Lake Reservoir. St. Mary Reservoir, Forty-Mile Coulee Reservoir, and Sherburne Lake are part of the St. Mary River Irrigation District. McGregor, Travers, and Little Bow are all part of the Carseland-Bow River Headworks system. (Table 4.1; Figure 4.1).

At each reservoir, we collected: fish, aquatic invertebrates, macrophytes, periphyton, native snails and clams, and where available, *C. chinensis* and *F. virilis*. To collect large-bodied

fish species, such as Walleye (*Sander vitreus*) and Northern Pike (*Esox lucius*), we used multi-mesh gillnets (7.62 m L x 1.83 m W, with stretched mesh sizes of 25, 38, 51, 64, 76, 102, 127, and 152 mm, respectively, there are no spacers between the mesh panels), left at randomized locations and depths in the lake for a maximum of 12 hours. To collect the small-bodied native species, such as Spottail Shiner (*Notropis hudsonius*) and Yellow Perch (*Perca flavescens*), we used two survey methods to maximize the chance of success in catching the required quantity of each species; backpack electrofisher and beach seining. Beach seining was completed in 100 m efforts, and repeated until at least 10 individuals of each species were caught. If not, backpack electrofishing (Smith-Root LR24 backpack unit) was done in 100 m efforts, along accessible areas on the shore. Kick net sampling for aquatic invertebrates was conducted at three separate locations around the lake in accessible areas, at 0 m, 0.5 m, and 1 m depth perpendicular to the beach. Simultaneously, macrophytes were collected at these three spots and depths. Periphyton was scraped off rocks using a toothbrush and placed directly into 2.5 mL microcentrifuge tubes. Native snails and clams were collected during 2-hour snorkel search efforts, or until at least 10 snails and 10 clams were collected. At McGregor Lake, *C. chinensis* were collected upon incidental observation and 2-hour search efforts in areas where *C. chinensis* had been previously confirmed to occupy (Edgar et al. 2022). Where *F. virilis* could be found, traplines with 5 modified minnow traps baited with Whiskas® Wet Cat Food were set at five different locations, at least 50 m apart, per lake and left overnight for 24 hours. If the quota for at least 10 individuals was not captured the first 24 hours, traplines were reset. At St. Mary's, despite no historical reports of *F. virilis*, we set overnight traplines for three consecutive days to confirm absence of species. All organisms were placed on ice and frozen immediately following collection, and transported to the University of Alberta where they were kept frozen at -20°C.

2.2. Lab analysis

In the lab, kick samples were thawed and individual invertebrates were identified to taxonomical order or family, if all anatomical identifiers were present (Clifford 1991). Individuals of the same taxonomical rank at each site were pooled to ensure a minimum of 0.4 mg of freeze-dried homogenized tissue for the mass spectrometer. All macrophytes were identified to species (Government of Alberta, 1989). Muscle tissue was collected from *F. virilis* for isotope analysis. Foot tissue from *C. chinensis* and dorsal tissue from fish were retained for stable isotope analysis. All plant and invertebrate tissues, *F. virilis*, *C. chinensis*, and fish tissues were then freeze-dried for 24 hours at -54°C at 0.015 Barr in a LABCONCO® Freezone 1 Liter Benchtop Freeze Dry System (Labconco, 2021). After freeze-drying, samples were homogenized using a mortar and pestle in a sterile environment, with tools cleaned and disinfected in between samples to retain sample integrity. Homogenized tissues were weighed to 0.4000 - 0.4999 mg into 6 mm x 8 mm tin capsules using a UMX2 Ultra-Microbalance (Mettler and Toledo, 2004). Every 13th sample was a replicate of the first sample for standardization and quality control. Samples were analyzed using a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) (Thermo Scientific Inc., Bremen, Germany). The IRMS is used to flash combust samples and convert them into gases, which are then separated using chromatography. The intensities of the heavier isotopes of ^{13}C and ^{15}N were compared to the international reference scales of vPDB and Air, respectively. Finally, isotope values are reported in the standard delta notation, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for analysis.

2.3. Statistical Analyses and Modelling

2.3.1. Preliminary data investigation

Prior to analyses, the collected data were reformatted for use in food web analyses. Fishes were grouped into functional feeding groups (e.g., general invertivore, piscivore, benthic invertivore/molluscivorous), based on literature and predicted seasonal diet type (Amundsen and Sánchez-Hernández 2019; Doll and Jacquemin 2021).

Lipids have varying degrees of carbon turnover and tend to have lower $\delta^{13}\text{C}$ values in comparison to carbohydrates and proteins. Therefore, I checked for the effects of inorganic carbon and lipids on the data and any variability in benthic invertebrate data points to avoid bias in our study (Silberberger et al. 2021). Samples with a C:N ratio higher than 4.0 were those that required lipid correction (Post et al. 2007; Hoffman and Sutton 2010). Samples with problematic C:N ratios occurred only among Lake Whitefish samples. I used a mathematical lipid correction for fish muscle tissue (Equation 1), because chemical lipid removal risks affecting $\delta^{15}\text{N}$ values, are proven to work as effectively as physical correction, and requires the preparation of duplicate samples (Silberberger et al. 2021).

Equation 1:

$$\delta^{13}\text{C lipidcorrection} = \delta^{13}\text{C} + 7.018 * \left(\frac{\frac{0.048 + 3.9}{1 + 287}}{9.3} \right)^{-1} \frac{1}{1 + 0.246 * \text{C:N} - 0.776}$$

2.3.2. Selecting isotopic baselines and calculations

In order to choose a baseline and appropriate end-member for stable isotope analysis, I analyzed functional feeding groups of invertebrates among all five reservoirs.

Kruskal-Wallis rank sum test was used to determine if there were any differences between functional feeding groups of invertebrates, as the data were non-normally distributed (Kruskal-Wallis chi-squared = 39.108, df = 12, p-value = 0.000101, p<0.05). Pairwise comparisons (Dunn test with Hochberg adjustment) and visual assessments of iso-space plots were conducted, which resulted in no significant different differences between means of primary consumer feeding groups. Additionally, patterns were inconsistent among reservoirs, therefore all primary consumers (trophic level 2) excluding suspected predator species, were used to calculate the baseline for all reservoirs.

Following the protocol outlined by Anderson and Cabana (2007), baseline $\delta^{15}\text{N}$ were calculated and then used to calculate trophic position (TP), a function of raw $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (Equation 3). Corrected $\delta^{13}\text{C}$, $\delta^{13}\text{C}_{\text{corr}}$, was also calculated using baseline $\delta^{15}\text{N}$ values (Equation 2).

2.3.3. $\delta^{13}\text{C}$ Correction

$\delta^{13}\text{C}$ values for consumer species were corrected using the following equation (Olsson et al. 2009).

Equation 2:

$$\delta^{13}\text{C}_{\text{corr}} = \frac{(\delta^{13}\text{C}_{\text{con}} - \delta^{13}\text{C}_{\text{ubaseline}})}{\text{CR}_{\text{baseline}}}$$

2.3.4. Trophic Position

Trophic position was calculated for each consumer in each reservoir system using the following equation for the single source trophic position model as outlined by Post (2002):

Equation 3:

$$TP_{con} = \lambda + \frac{(\delta^{15}N_{con} - \delta^{15}N_{base})}{\Delta n}$$

Where TP_{con} is the trophic position of the consumer/target species in its respective reservoir, λ is the trophic position of baseline organisms ($\lambda=2$ for non-predatory, herbivorous benthic invertebrates), $\delta^{15}N_{con}$ is the raw isotopic nitrogen value of an individual consumer, $\delta^{15}N_{base}$ is the calculated baseline $\delta^{15}N$ value, and Δn is the trophic enrichment factor (TEF) equal to 3.4‰ (SD = 1‰). The 3.4‰ TEF value is widely used as a proxy for the trophic step from primary consumers to predatory species (Silberberger et al. 2021). This TEF is a valid approximation for the studied ecosystems, as it was developed largely on both pelagic and littoral species, and suitable for other primary consumers in the ecosystems, as described by Post (2002).

2.3.5 Isotopic Niche and Food-web

The isotopic food web of each reservoir was analyzed using the Stable Isotope Bayesian Ellipses in R (SIBER) (Jackson et al. 2011) and used to calculate Bayesian Layman metrics. $\delta^{15}N$ range, $\delta^{13}C$ range and mean distance to centroid (CD) are measures used to analyze the structure of the food web within the $\delta^{15}N/\delta^{13}C$ scatterplot space. $\delta^{15}N$ range is a proxy for the length of the food web, whereas the range of $\delta^{13}C$ values reflects the trophic diversity at the base of the food web (Saporiti et al. 2015). Distance to centroid (CD) estimates the average degree of trophic diversity

within each community's food web and its redundancy (Micheli and Halpern 2005; Saporiti et al. 2015). Mean nearest neighbour distance (MNND) and the standard deviation of nearest neighbour distance (SDNND) reflect the relative positions of the species to each other within the community iso-space and may also be used as a proxy to estimate the extent of trophic redundancy (Layman et al. 2007; Saporiti et al. 2015).

Due to the package sample size requirements, samples where there were less than three individuals in a group were removed. This resulted in the removal of 7 samples. Isotopic niche of select species within and between lake reservoir systems were analyzed using SIBER (Jackson et al. 2011).

The SIBER model used to calculate Bayesian community metrics was run with the following parameters to ensure model convergence: 10^4 iterations, 10^3 discard values, 10 thinned posteriors, with 2 chains. SEA_b modes and SEA_b credible intervals were calculated (Bayesian calculated standard ellipse area modes and credible intervals) (Table 2).

I calculated both standard ellipse area corrected for small sample sizes (SEA_c) and total area (TA) of both *C. chinensis* and *F. virilis* (Table 3).

Within McGregor Lake, *C. chinensis* niche was compared to *F. virilis* niche, to investigate any interaction or niche overlap between these two invasive species.

2.3.6. *Mixing Models*

Individual mixing models were run separately for each lake reservoir, using the MixSIAR package for R (Stock et al. 2020). The package creates and runs Bayesian mixing models to analyze $\delta^{15}N$ and $\delta^{13}C$ to estimate proportions of source (prey) contributions to a mixture (consumer species). One key assumption that must be made for Bayesian mixing models is the

selection of trophic enrichment factors (TEFs) (Bond and Diamond 2011). As was previously used for the calculation of trophic position values, we assumed a trophic fractionation of $3.4 \pm 1\text{‰}$ for $\delta^{15}\text{N}$ for each trophic step (Post 2002). For $\delta^{13}\text{C}$, a trophic fractionation factor of $0.4 \pm 1.3\text{‰}$ for each trophic step was used (Post 2002). Models converged with a chain length of 100,000, a burn in of 50,000, a thinning rate of 50, and 3 chains using the Dirichlet distribution and an uninformative prior, which considers that all combinations of sources are equally likely to occur (Stock and Semmens 2020). The choice to use the Dirichlet distribution was made because although fish diets are generally known, quantitative diet composition was lacking for our systems. Residual and process error, to account for variation in consumer tracer values due to sampling process and agents of consumer variability (Stock and Semmens 2016), respectively, were included in each reservoir's mixing model. We confirmed model convergence using Gelman-Rubin diagnostics (Gelman and Rubin 1992). Consumer species selected were recreational, consumer fish species: Walleye, Northern Pike, and Lake Whitefish. Based on reviews of our recreational fish species' diets and available organisms during sampling, sources were listed as any potential prey source (*C. chinensis*, *F. virilis*, macrophytes, benthic macroinvertebrates, periphyton, Spottail Shiner, and Yellow Perch) (Amundsen and Sánchez-Hernández 2019; Doll and Jacquemin 2021).

2.3.7 Stomach content analysis

Retained fish were collected whole, frozen immediately, and brought to the lab for both dissection of muscle tissue, as well as stomach content analysis. The purpose of conducting stomach content analysis was to better inform diet of collected consumer species, and determine if they are consuming *F. virilis* and *C. chinensis*. Fish were thawed and stomachs were removed

and placed in water-diluted ethanol (80:20, ethanol:water) to preserve contents but not cause breakdown of identifiable features of invertebrates. The following metrics were collected for each stomach: blotted wet weight (bww) of entire stomach, bww of item groups, count of items belonging to each group, % fullness, and identification of item groups to class or family.

Unidentifiable organic matter was categorized as seston. However, due to the high proportion of empty stomachs, stomach content dissections were only used to provide evidence regarding the consumption of invasive species by consumer fish species. The frequency of occurrence of a prey item equaled the number of times a prey item occurred in the fish species examined divided by the total number of stomachs analysed for that species in a specific reservoir. Frequency of items was then compared between treatments to assess whether diet items changed significantly.

3. Results

3.1. Catch data

A total of 868 samples were collected among Forty-Mile, McGregor, Sherburne, St. Mary, and Travers Lake reservoirs. The quantities of each fish species, individuals within invertebrate functional feeding groups, macrophyte and periphyton samples per lake are provided (Appendix; Table 1).

3.2. Trophic Position

The result from the Kruskal-Wallis's chi-squared test were significant when analyzing differences of TP across treatment types ($H=30.291$, $df=2$, $p\text{-value}=2.645*10^{-7}$). Values differed significantly between the sympatric reservoir and control ($p\text{.adj}=0.006$), and sympatric reservoir and *F. virilis* only reservoirs ($p\text{.adj}=0.000000115$) but not between the control and *F. virilis* only reservoirs ($p\text{.adj}=0.882$). Similarly, a Kruskal-Wallis chi-squared test comparing differences of $\delta^{13}\text{C}_{\text{corr}}$ values across treatments was significant ($H=21.914$, $df=2$, $p\text{-value}=1.743*10^{-5}$). A

Dunn's test pairwise comparison test resulted in $\delta^{13}\text{C}_{\text{corr}}$ values that were significantly different between the sympatric reservoir and control ($p.\text{adj}=0.000131$) and *F.virilis* reservoirs and control ($p.\text{adj}=0.0000203$) but not the sympatric reservoir and *F. virilis* only reservoirs ($p.\text{adj}=1$) (Figure 4.3).

Northern Pike in systems with only one invasive species present have significantly higher mean trophic position than Northern Pike in systems with both invasive species ($p.\text{adj}= 0.00123$) However, there are no significant differences between Northern Pike $\delta^{13}\text{C}_{\text{corr}}$ values between reservoirs (Figure 4.4).

Lake Whitefish demonstrated significant differences in mean trophic position between systems with both invasives and with only *F. virilis* ($p.\text{adj}= 0.00656$), and the control reservoir and systems with just one invasive ($p.\text{adj}= 0.0000197$). Systems with just *F. virilis* had a higher mean trophic position when compared to the system with co-occurring invasives, and the control group had the lowest mean trophic position. Significantly higher differences in mean $\delta^{13}\text{C}_{\text{corr}}$ values occur in systems with only one invasive compared to the system with both invasives ($p.\text{adj}= 0.0000444$). The Lake Whitefish in systems with one invasive species, *F. virilis*, have a significantly higher mean $\delta^{13}\text{C}_{\text{corr}}$ value compared to the control system ($p.\text{adj}=0.00000000426$). (Figure 4.4).

Walleye had significantly higher mean trophic positions in systems with only one invasive species, compared to systems with both ($p.\text{adj}=7.68\text{e- }5$). Similarly, Walleye in systems with just one invasive species also had significantly higher mean trophic position than the Walleye in the control system ($p.\text{adj}= 2.80\text{e-}11$). There are significant differences between

$\delta^{13}\text{C}_{\text{corr}}$ values ($H = 8.4888$, $df = 2$, $p\text{-value} = 0.01434$) at the 5% level, however there were no differences between groups using a Dunn-test. (Figure 4.4).

F. virilis have significantly different mean trophic positions between systems where it occurred alone and where it occurred with *C. chinensis* ($p_{\text{adj}} = 0.0377$). However, no significant difference in $\delta^{13}\text{C}_{\text{corr}}$ values exists between either treatment where *F. virilis* occur.

3.3. Comparing community trophic structure and trophic positions

On average, the systems with just one invasive species are lower in all the estimated community metrics except in $\delta^{15}\text{N}$ range, which is higher. The range of $\delta^{15}\text{N}$ is a proxy for “food web length”. Contrary to our hypothesis, reservoirs with just *F. virilis* had wider food web lengths than the systems with *C. chinensis* and *F. virilis* in sympatry. The control reservoir, St. Mary’s Reservoir, had the lowest $\delta^{15}\text{N}$ range (3.0‰), followed by the lake with both invasives (3.3‰), and then the systems with only crayfish having the highest $\delta^{15}\text{N}$ range (4.1‰). Generally, a larger range in $\delta^{15}\text{N}$ indicates more trophic levels and a greater trophic diversity (Post 2002)

The reservoir with sympatric invasives have the highest $\delta^{13}\text{C}$ range (2.5‰), comparatively to the control with second largest carbon range (2.1‰) and the reservoir with only *F. virilis* which had the lowest carbon range (1.8‰).

With the addition of invasive species, the total area (TA), a proxy for the total extent of trophic diversity within the food web, increased from an average of 2.4‰², 2.7‰², and 3.0‰², for the control, *F. virilis* only reservoirs, and sympatric reservoir, respectively. Distance to

centroid (CD) was ultimately similar between all three treatments, with the control and sympatric reservoir having an average CD value of 1.0‰ and the *F. virilis* reservoirs having a CD value of 0.9‰.

However, nearest-neighbour-distance (NND) was highest in the control reservoir (0.7‰), and similar between the *F. virilis* only reservoirs and sympatric reservoir (0.4 and 0.5‰, respectively). (Table 4.2).

3.4. Comparing isotopic niche between *C. chinensis* and *F. virilis* in McGregor Lake Reservoir

Due to McGregor Lake Reservoir being the only waterbody where *C. chinensis* and *F. virilis* are sympatric, this is the only lake for which I calculated niche overlap and standard ellipse areas. *C. chinensis* had an SEA_c of 0.14‰^2 and *F. virilis* had a SEA_c of 0.16‰^2 (Table 4.3; Figure 4.6). The niche overlap between corresponding 95% prediction ellipses was calculated at 61.4% overlap (Figure 4.5). Based on the Bayesian proportion calculations and posterior draws for both groups, *C. chinensis* are predicted to have a 66.25% probability of having a smaller SEA_b compared to *F. virilis*. Total area for *C. chinensis* was calculated as 0.45‰^2 , whereas *F. virilis* in the same waterbody have a larger total area of 0.73‰^2 .

3.5. Mixing Models: Determining diet source proportions of consumers in each reservoir

MixSIAR results for each *reservoir + fish species + source* combination and confidence intervals (%), mean, and standard deviation values are presented in Appendix; Table A4.3.

All three recreational fish species (Lake Whitefish, Northern Pike, and Walleye) utilize macrophytes as a diet item in the control reservoir (81%, 40.9%, and 31.2%, respectively). In invaded lakes, macrophytes contribute much less to diets. In McGregor Lake Reservoir, macrophytes contributed 2.2, 4.2, and 0.9%, respectively, of Lake Whitefish, Northern Pike, and Walleye diets. In Travers Lake Reservoir, macrophytes contributed 0.4, 1.7, and 0.6%, respectively, of Lake Whitefish, Northern Pike, and Walleye diets. In Sherburne Reservoir, macrophytes contributed 3.0, 2.9, and 1.0%, respectively, of Lake Whitefish, Northern Pike, and Walleye diets. In Forty-Mile Reservoir, macrophytes contributed to 1.1, 0.5, and 0.3%, respectively, of Lake Whitefish, Northern Pike, and Walleye diets (Appendix; Table 4.3).

Secondary consumers comprised a majority of the diet of Walleye and Northern Pike but did not contribute as much to Lake Whitefish diet in the control reservoir (66.3, 52, and 12%, respectively). However, in lakes where invasive species are present, the proportion of Lake Whitefish diet attributed to secondary consumers, such as Spottail Shiner, increased to 83.7% in McGregor Lake and 24.9, 77.4, and 59.1% for Travers, Sherburne, and Forty-Mile Reservoirs, respectively. Though not as extreme, Northern Pike and Walleye also had slight increases in the secondary consumer inputs to diets. In McGregor Lake, secondary consumers contributed 92.4 and 49.3% of Walleye and Northern Pike diets. In the reservoirs with only *F. virilis* present, Walleye diets were comprised of 84.6, 91.6, and 93.2% secondary consumers (Travers, Sherburne, and Forty-Mile Reservoir). Northern Pike were more conservative, and did not increase to the same extent, with 50.2, 75.2, 84.6% of their diets attributed to secondary consumers in Travers, Sherburne, and Forty-Mile Reservoir.

In reservoir systems where there is only *F. virilis* present as an invasive species, the three main recreational species are utilizing *F. virilis* as a food item at a higher proportion than when *F. virilis* exists in sympatry with *C. chinensis*. In Travers Lake Reservoir, *F. virilis* contributed 2.5, 15.4, and 3.6%, respectively, of Lake Whitefish, Northern Pike, and Walleye diets. In Sherburne Reservoir, *F. virilis* contributed to 10.2, 14.8, and 3.5%, respectively, of Lake Whitefish, Northern Pike, and Walleye diets. And, in Forty-Mile Reservoir, *F. virilis* contributed to 5.5, 3.1, and 1.7%, respectively, of Lake Whitefish, Northern Pike, and Walleye diets. Then, in the sympatric reservoir, *F. virilis* contributed to 1.4, 5.7, and 1.2%, respectively, of Lake Whitefish, Northern Pike, and Walleye diets. *C. chinensis* contributed to 1.4, 6.0, and 1.3%, respectively, of Lake Whitefish, Northern Pike, and Walleye diets (Appendix; Table A4.3).

3.6. Gut content analysis

Of the 283 stomachs that were collected from fish samples, empty stomachs occurred in 40.6% of the fishes. *F. virilis* were not a part of stomach contents in Forty-Mile or Sherburne Lake Reservoirs. However, *F. virilis* in Lake Whitefish stomachs in Travers reservoir comprised 2.5% of their diets, and 26% of Northern Pike diets. In McGregor Lake Reservoir, *F. virilis* comprised 50% of Northern Pike diets, and 14% of Walleye stomach contents. *C. chinensis* did not occur in any of the analyzed stomachs.

4. Discussion

The overlap of *C. chinensis* and *F. virilis* trophic niche ellipses within McGregor Reservoir suggest competitive exclusion (Meszéna et al. 2006), and to a lesser degree *F. virilis* alone, may be leading to the decline of macrophyte availability, and alteration of fish diets.

Changes in macrophyte availability in lakes and reservoirs have the potential to affect the physical (light extinction, temperature, substrate, hydrodynamics) and chemical environments (oxygen, inorganic and organic carbon, nutrients) and biota (Carpenter and Lodge 1986). Bottom-up changes caused by a decrease in macrophyte biomass include declining fish recruitment (Breck and Kitchell 1979), which ultimately leads to an overall decrease in ecosystem productivity (Fontaine and Ewel 1979; Carpenter and Lodge 1986). In addition, the formerly colonizable surface of macrophytes used by native snails would disappear with a reduction of macrophytes (Carpenter and Lodge 1986).

Since it is relatively early in the introduction and establishment of *C. chinensis* in McGregor Lake Reservoir, crayfish may directly and indirectly limit macrophyte biomass, thus competing directly for diet items used by *C. chinensis*. Both consumptive and non-consumptive effects caused by interactions between *C. chinensis* and *F. virilis* may lead to competitive exclusion if the tolerable niche overlap is exceeded (Pianka 1974). This could explain the overlapping isotopic niche space and absence of macrophytes in the diets of recreational fish. Snails are also known to forego their preferred rock substrate with high periphyton abundance for less desirable macrophytes in the presence of crayfish (Weber and Lodge 1990). Further, after the initial introduction of *C. chinensis* into waterbodies, native snails will eat much more than what is normal for native snails, having far reaching effects on the entire food web (Sura and Mahon 2011). As suggested by their overlapping niches and because *F. virilis* have a larger total niche area, *C. chinensis* may be outcompeted or used as a food source, exacerbating the issue of *F. virilis* as an aquatic invader, as has happened in Washington, U.S.A. (Olden et al. 2013).

Changes in fish trophic position would be expected if there were shifts in diet items (Hoeinghaus and Zeug 2008), which occurred in the study reservoirs where only *F. virilis* was present. Counter to this, the reservoir with both invasive species present shared similar trophic ranges and average species-specific trophic positions as the uninvaded waterbody. Because CD is a measure of average trophic diversity in the food web, and because all treatments are similar, the trophic diversity may be similar between the treatment types. Reservoirs with higher NND, the measure for overall density of species packing in an isotopic space, would indicate a smaller number of species with similar trophic ecologies existing in the same ecosystem (Layman et al. 2007). (Table 4.2).

The changes in $\delta^{13}\text{C}$ range between invasion levels indicates shifts in the sources of organism nutrition in these waterbodies (Vander Zanden et al. 1999), as carbon ranges widen when both invasives are present but narrow when only one, *F. virilis*, is present. Increased $\delta^{13}\text{C}$ range is expected in food webs where there are multiple basal resources present (e.g., *C. chinensis* and *F. virilis* in sympatry), which results in niche diversification at the base of the food web. This may also be due to fish consuming less macrophytes where invasives are present, diversifying their diets for nutrients they require but cannot acquire from macrophytes.

Reasons for fish consuming less macrophytes when invasives are present may be due to the addition of invasive species that are both known to cause declines in aquatic vegetation biomass. Crayfish destroy macrophytes (Carpenter and Lodge 1986). *C. chinensis* are consumers of macrophytes and in larger densities are known to lead to collapses in macrophyte assimilation in waterbodies (Kingsbury et al. 2021). Individually, the presence of these two invasives are

also known to alter the behavior of native snail species (Sura and Mahon 2011). In my research systems, native species may be reallocating energy in the presence of competition (with *C. chinensis*) or predation (from *F. virilis*) by expending more energy on locating food as a result of these interactions (Lewis et al. 2001; Cross and Benke 2002; Johnson et al. 2009), as demonstrated by shifts in trophic positions. The presence of both *C. chinensis* and *F. virilis* may also be leading to a decline in available macrophytes, in reservoirs where they are present. Lake Whitefish, Northern Pike, and Walleye all consume significantly less macrophytes when invaders are present (Appendix; Table A4.3), suggesting that macrophytes are not present in dense enough volume to be used as a diet item due to resource limitation caused by invasive crayfish on *C. chinensis*.

Shifts in the diets of recreational fish due to the presence of aquatic invasive species may result in increased competition between species and avian piscivores (Pothoven and Madenjian 2013). Both Walleye and Northern Pike showed changes in diet and trophic position with the addition of only one invasive species into the ecosystem. However, with the introduction of *C. chinensis*, Northern Pike had a shift towards a lower trophic position. Conceivably, this may be from a lesser proportion of their diet consisting of *F. virilis* and a shift in the basal diet contribution. Walleye diets were significantly different with the addition of only *F. virilis*, however when *C. chinensis* was added to the ecosystem, both trophic position and carbon range were quantitatively lower. Shifts in diet create changes to trophic position and carbon range, which may be due to lack of macrophytes and competitive exclusion between *C. chinensis* and *F. virilis* when they are in sympatry. Walleye and Northern Pike do not historically actively prey on mollusks (Diana 1979; Sheppard et al. 2015). However, a learning period might be needed for predators to adapt to novel prey like *C. chinensis* (Brownscombe and Fox 2013). Further, when

at least one invasive species was added to the ecosystem, Lake Whitefish shifted from consuming macrophytes to primary and secondary consumers, which created a shift towards a higher trophic position as a result of increased piscivory. Shifts to piscivory in Lake Whitefish have been associated with declines of body condition and growth and reduced energy content of the diet when aquatic invasive species are added to the ecosystem (Rennie et al. 2009), which has happened with the addition of Round Goby (*Neogobius melanostomus*) in Lake Huron and the Sydenham River (Firth et al. 2021) and dreissenid mussels in the Great Lakes (Pothoven and Madenjian 2013).

Though Walleye, Northern Pike, and Lake Whitefish may not be consuming *C. chinensis* or *F. virilis* in larger proportions, and their diets are switching to more dominant piscivory in the presence of invasives, there may be an “adaptive lag” period before they begin preying heavily on these invasive species (Carlsson et al. 2009; Brownscombe and Fox 2013).

5. Further comments

Impacts of aquatic invasive species on fish are potentially muddled by context-dependencies such as nutrient enrichment in lakes and reservoirs (Catford et al 2021), which modulate the trophic niche (Almeda et al 2021) resulting in different impacts among invaded lake systems (Boets et al 2021; Tatenda et al 2022). The reservoir systems in this study were in very close proximity, if not adjacent, to agricultural lands. The potential for nutrient enrichment from fertilizers and farming activities is very high, and with the last dissolved nutrient measurements taken in 2013 and 2014, there is a chance that trophic level approximates could be altered or interpreted incorrectly. Additionally, reservoirs and lakes in Alberta are suffering from drought, due to stochastic weather events and increasing pressure from the agricultural industry.

Stable isotopes only provide information on the flow of energy or nutrients throughout a food web and do not offer definitive information when it comes to functional relationships between organisms (Layman et al. 2012). There may also be non-consumptive effects of crayfish on snails, mediated by trait changes, which could potentially alter their populations and may not be entirely described by stable isotope analysis. One mechanism is by limiting the amount of available habitat and restricting snails to vertical or near-surface refuges, consequently decreasing carrying capacity (Lewis et al. 2001; Dorn 2013). This would be further impacted by the extreme varying water levels that occur in reservoir systems, especially if snails are limited to near-surface refuges. Also, non-consumptive interactions could lead to limited snail populations if crayfish induce snails to invest in thicker shells, rapid growth, or costly migrations to and from a refuge resulting in reduced reproductive effort (Dorn 2013).

Long-term collection of *C. chinensis* samples and habitat data for future occupancy modelling of this species should be considered as not much is known about where this species can successfully colonize in central and western Canada (Kingsbury 2020). Further fine-scale investigation into the effects of *C. chinensis* on basal resources should be conducted, as this study did not cover the scope needed to properly conclude the effect on native snail species and the bottom-up effects that impacts to them might have. Further work should also assess relative predation risk to *C. chinensis* and *F. virilis* in lotic waterbodies, as this may be a contributing factor to their range expansion. Other work could also investigate fish body condition and energetics with the addition of these invasive species, especially on Lake Whitefish as they are diet switching towards piscivory.

6. Conclusion

Community trophic structure in southern Alberta has changed because of the range expansion of *F. virilis* and addition of non-native *C. chinensis* and resulting competitive exclusion. Native recreational fish species are minimally consuming *C. chinensis*, however they consume *F. virilis* at higher proportions in waterbodies where they are the sole aquatic invasive macroinvertebrate, increasing recreational fish species trophic positions. Both *C. chinensis* and *F. virilis* are exhibiting bottom-up trophic effects on fish species in invaded waterbodies by contributing to the absence of macrophytes in diet, which may point to further major effects on aquatic ecosystems. For now, *C. chinensis* and *F. virilis* are competing for resources, and in turn this may be playing a part in limiting the amount of isotopic packing that can occur when additional aquatic invasive species are added to a waterbody, as shown in reservoirs where *F. virilis* exists allopatrically from *C. chinensis*.

Research regarding *C. chinensis* and *F. virilis*' potential effects in sympatry, in large and variable systems, have been lacking. This research provides an insight to the effects of these two species when they exist together, as well as the effects of *F. virilis*, in reservoir ecosystems in southern Alberta. This research also provides a baseline for future research that should be carried out if *C. chinensis* is to persist, as is anticipated. Management of *C. chinensis* should focus on long-term containment in McGregor Lake Reservoir and monitoring of the effects this species will have on native species, particularly native snails and macrophyte biomass, if it continues to persist in this location.

Tables and Figures

Table 4.1: List of locations of each reservoir lake, surface area in hectares (ha), mean depth and maximum depth (m)(max.), total phosphorous (TP ($\text{mg} \cdot \text{L}^{-1}/\text{year}$)) and total nitrogen (TN ($\text{mg} \cdot \text{L}^{-1}/\text{year}$)).

	McGregor	Travers	Forty-Mile Coulee	Sherburne	St. Mary's
Latitude (°N)	50.42416	50.19515	49.64438	49.75645	49.36536
Longitude(°W)	-112.85145	-112.77083	-111.39697	-111.77751	-113.12415
<i>F. virilis</i>	Present	Present	Present	Present	Absent
<i>C. chinensis</i>	Present	Absent	Absent	Absent	Absent
Surface area (ha)	4902.88	2281.86	674.75	407.12	3527.02
Mean depth (m) (max.)	6.80 (12.04)	14.30 (38.67)	15.63 (25.48)	7.26 (16.66)	10.4 (56.4)
TP ($\text{mg} \cdot \text{L}^{-1}$)(year)	0.007 (2013)	0.012 (2013)	0.008 (2013)	0.032 (2014)	0.004 (2014)
TN ($\text{mg} \cdot \text{L}^{-1}$)(year)	0.226 (2013)	0.225 (2013)	0.373 (2013)	0.427 (2014)	0.107 (2014)

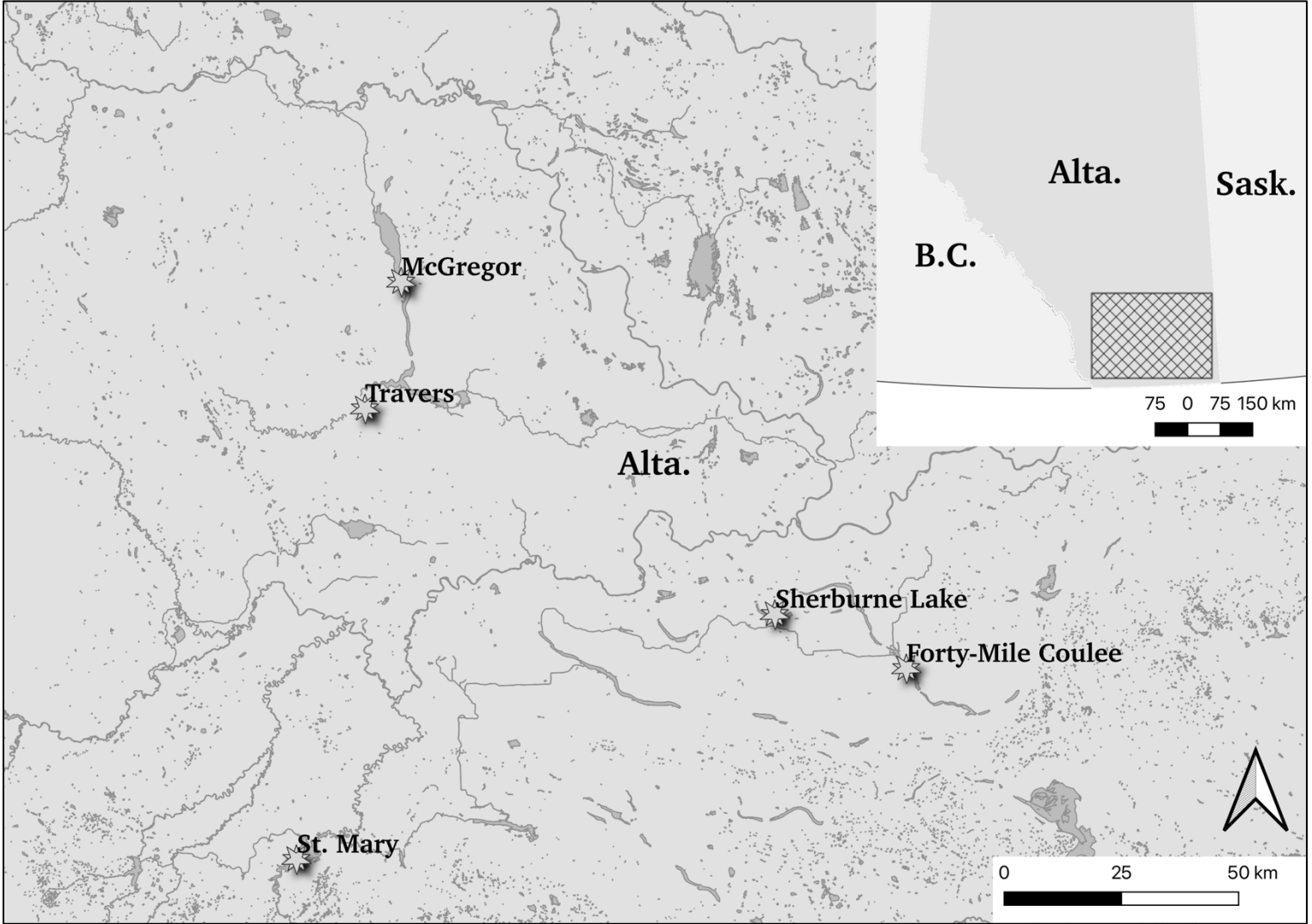


Figure 4.1: Map of study locations (McGregor Lake, Travers, St. Mary’s, Sherburne Lake, and Forty-Mile Coulee reservoirs) in Alberta (Alta.), Canada. Inset map shows study area (cross-hatched rectangle) relative to Alberta’s provincial boundaries shared by British Columbia (B.C.) and Saskatchewan (Sask.).

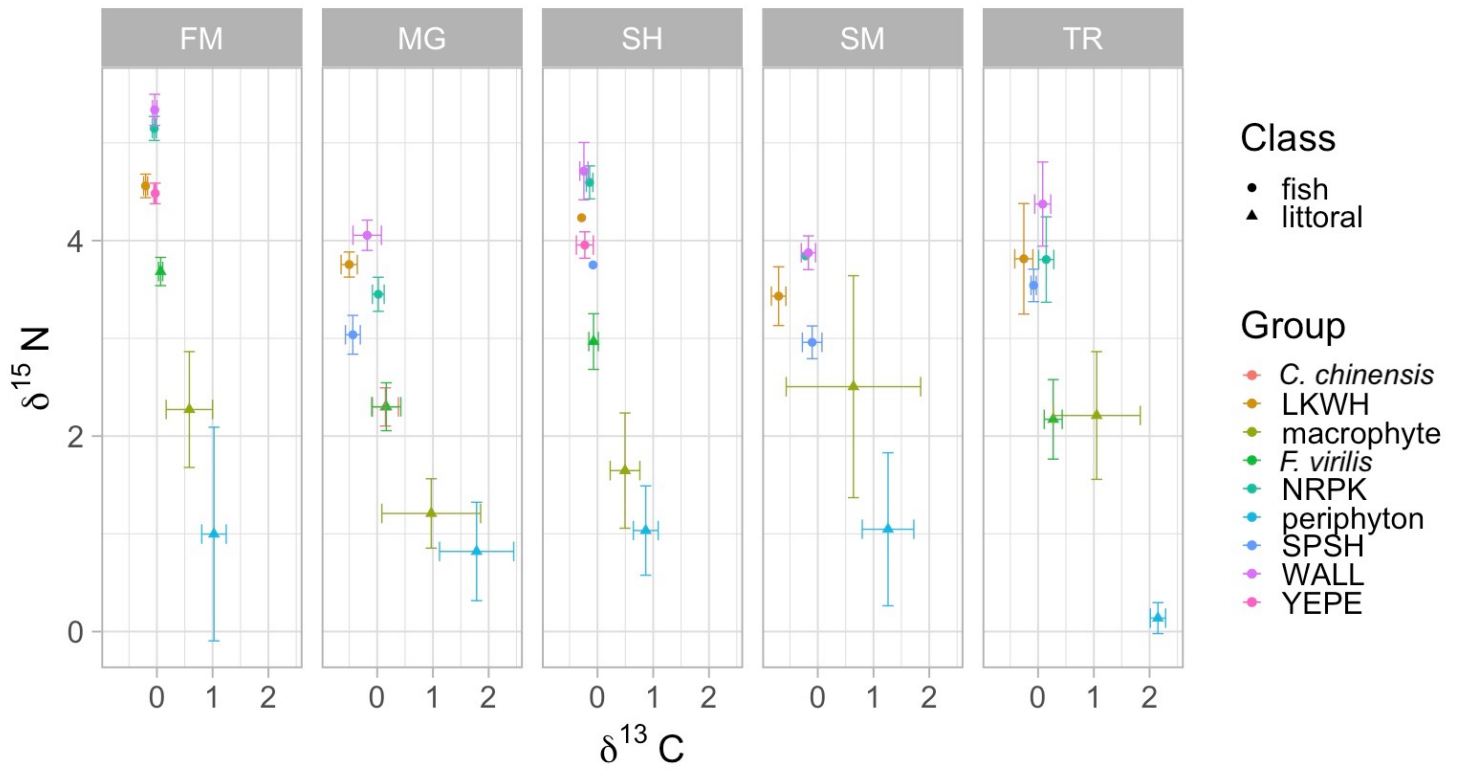


Figure 4.2: Iso-space plot of means and standard deviations for $\delta^{13}\text{C}_{\text{corr}}$ and baseline calculated $\delta^{15}\text{N}$ for each species within each reservoir. Reservoirs: MG= McGregor Lake Reservoir, FM= Forty-Mile Coulee Reservoir, SH= Sherburne Lake Reservoir, SM= St. Mary's Reservoir, TR=Travers Reservoir.

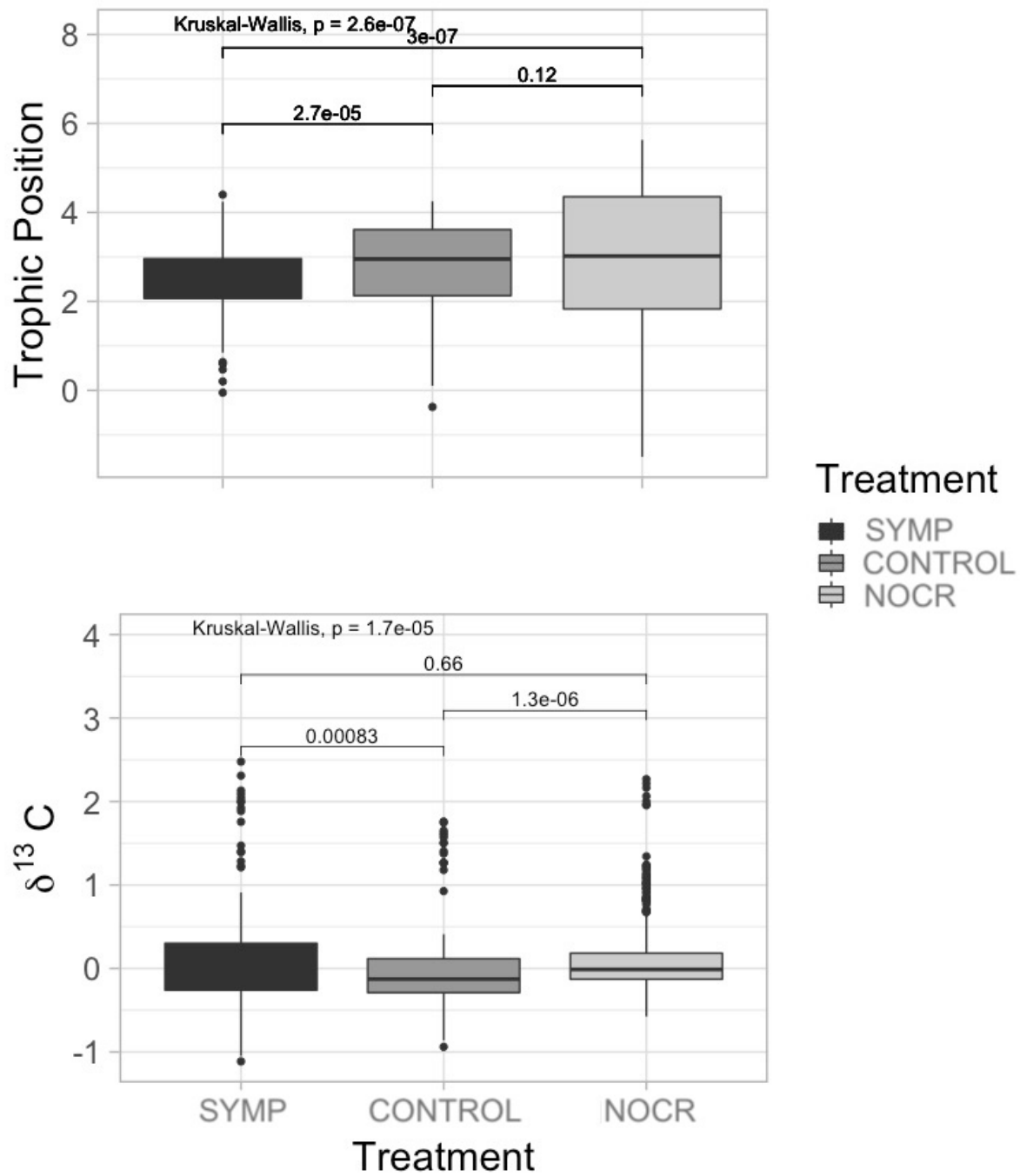


Figure 4.3: Box plots of the spread and mean of average trophic position (TP) and $\delta^{13}\text{C}$ in each treatment group, with accompanying pairwise comparisons (SYMP = reservoirs where *F. virilis* and *C. chinensis* are together (sympatry); CONTROL = both species absent (control), St. Mary's Reservoir; NOCR = Only *F. virilis* present).

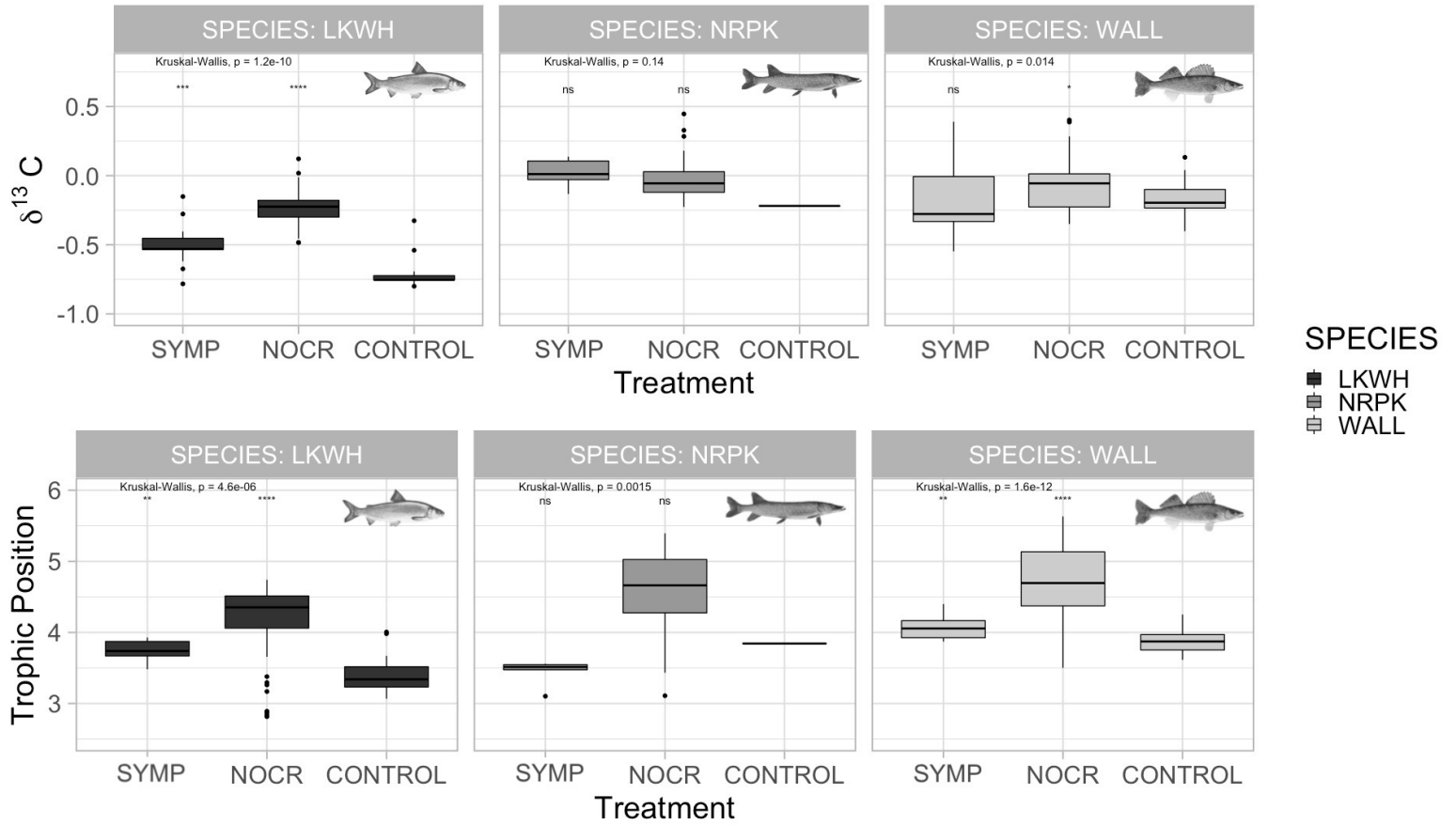


Figure 4.4: Box and whisker plots showing the spread of $\delta^{13}C_{corr}$ ($D_{13}C_{corrected}$) and Trophic position of each focus recreational species (LKWH = Lake Whitefish; NRPK = Northern Pike; WALL = Walleye) in all three treatment groups, with accompanying pairwise comparisons (SYMP = reservoirs where *F. virilis* and *C. chinensis* are together (sympatry); CONTROL = both species absent (control); NOCR = Only *F. virilis* present).

Table 4.2: Bayesian estimates of Total Area (TA) (%²), Distance to Centroid (CD) (%), carbon range (CR) (‰) and nitrogen range (NR) (‰) calculated for each system.

	Mode	Credible Intervals					
		99%		95%		50%	
		<i>lower</i>	<i>upper</i>	<i>lower</i>	<i>upper</i>	<i>lower</i>	<i>upper</i>
Forty-Mile Reservoir							
<i>(F. virilis)</i>							
δ^{15N} range	4.4	3.4	5.5	3.7	5.2	4.1	4.7
δ^{13C} range	1.5	0.7	2.5	0.8	2.2	1.2	1.7
TA	2.9	1.2	5.6	1.6	4.6	2.4	3.4
CD	1.3	1.2	1.6	1.2	1.5	1.3	1.4
NND	0.6	0.3	0.8	0.4	0.8	0.5	0.6
SDNND	0.4	0.2	0.6	0.2	0.6	0.3	0.4
Travers Lake Reservoir							
<i>(F. virilis)</i>							
δ^{15N} range	4.3	2.4	6.1	2.9	5.7	3.9	4.7
δ^{13C} range	2.7	1.0	4.6	1.4	4.0	2.2	3.1
TA	3.5	1.4	6.8	1.9	5.8	3.0	4.3
CD	1.2	0.9	1.5	1.0	1.4	1.1	1.3
NND	0.6	0.3	0.9	0.4	0.9	0.6	0.7
SDNND	0.6	0.1	1.3	0.2	1.1	0.5	0.8
Sherburne Reservoir							
<i>(F. virilis)</i>							
δ^{15N} range	3.7	2.9	4.7	3.1	4.4	3.5	4.0
δ^{13C} range	1.3	0.6	2.2	0.8	2.0	1.1	1.5
TA	1.8	0.7	3.4	1.0	3.0	1.5	2.2
CD	1.2	1.0	1.5	1.0	1.4	1.1	1.3
NND	0.5	0.3	0.7	0.3	0.7	0.4	0.6
SDNND	0.2	0.0	0.5	0.1	0.5	0.2	0.3
McGregor Reservoir							
<i>(C. chinensis and F. virilis)</i>							
δ^{15N} range	3.3	2.3	4.5	2.6	4.2	3.1	3.6
δ^{13C} range	2.5	1.4	3.7	1.7	3.4	2.2	2.8
TA	3.0	1.1	6.0	1.7	4.9	2.5	3.5
CD	1.0	0.8	1.3	0.8	1.2	1.0	1.1
NND	0.5	0.2	0.7	0.3	0.7	0.4	0.5
SDNND	0.4	0.1	0.6	0.1	0.5	0.2	0.4
St. Mary's Reservoir							
<i>(Control)</i>							
δ^{15N} range	3.0	1.6	5.7	2.0	4.4	2.7	3.4
δ^{13C} range	2.1	0.8	4.5	1.1	3.4	1.8	2.4
TA	2.4	0.4	8.1	0.7	5.3	1.9	3.0
CD	1.0	0.6	1.5	0.7	1.3	0.9	1.1
NND	0.7	0.3	1.2	0.4	1.0	0.6	0.7
SDNND	0.4	-0.1	1.3	0.1	0.8	0.3	0.5

Table 4.3: Calculated TA (Total Area) ($\%^2$), SEA (Standard Ellipse Area) ($\%^2$), and SEA_c (Standard Ellipse Area corrected for small sample sizes) ($\%^2$) of *F. virilis* and *C. chinensis* in each reservoir system.

	Forty-Mile Reservoir	Sherburne Reservoir	Travers Reservoir	McGregor Lake Reservoir	
	<i>F. virilis</i>	<i>F. virilis</i>	<i>F. virilis</i>	<i>F. virilis</i>	<i>C. chinensis</i>
TA	0.03	0.12	0.77	0.73	0.45
SEA	0.01	0.06	0.15	0.15	0.14
SEA_c	0.02	0.07	0.16	0.16	0.14

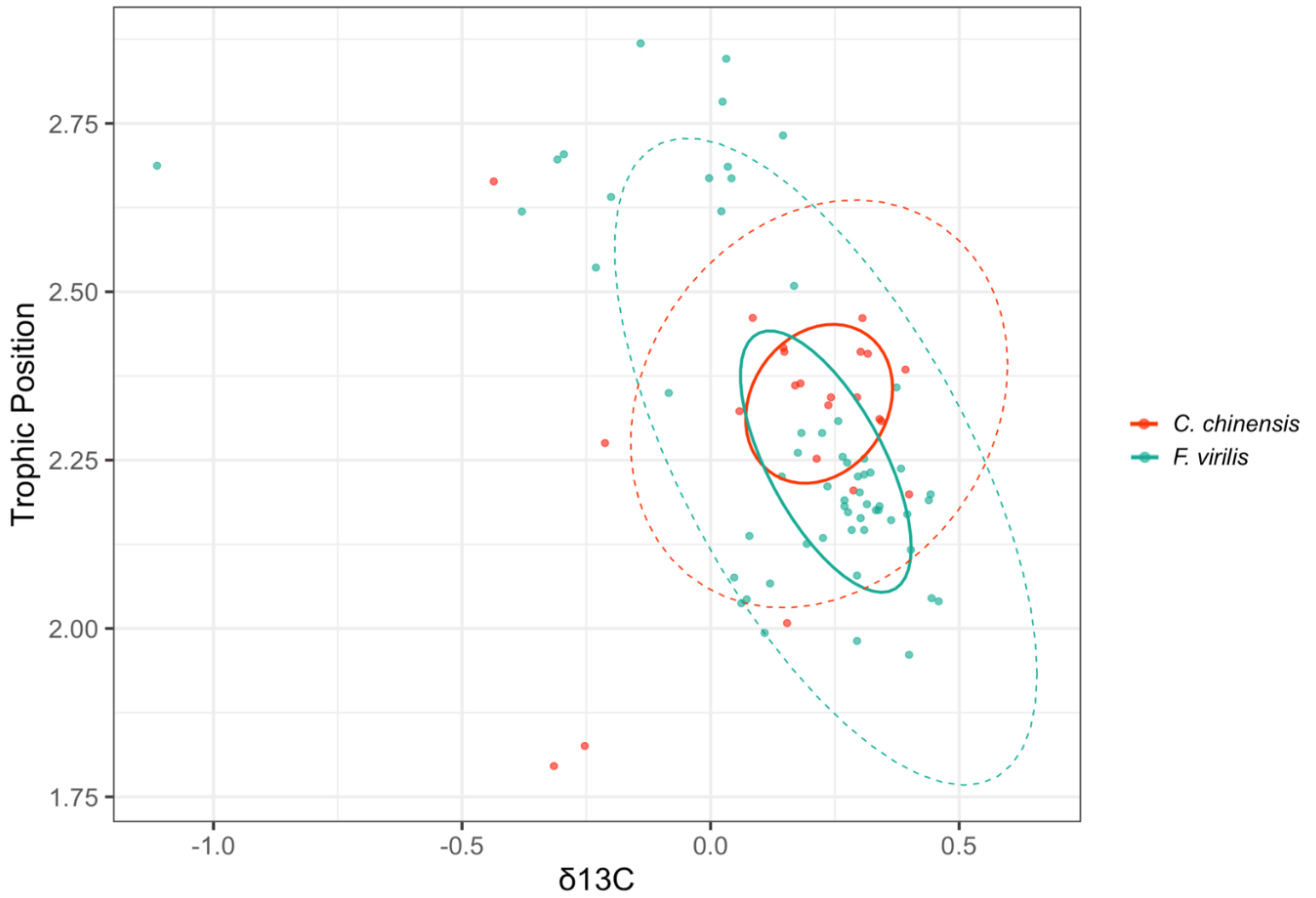


Figure 4.5: Bayesian standard ellipse areas of Northern Crayfish (*F. virilis*) and Chinese Mystery Snail (*C. chinensis*). Dashed lines represent the standard ellipse area and the solid lines represent the standard ellipse area (SEA) corrected for small sample sizes (SEA_c).

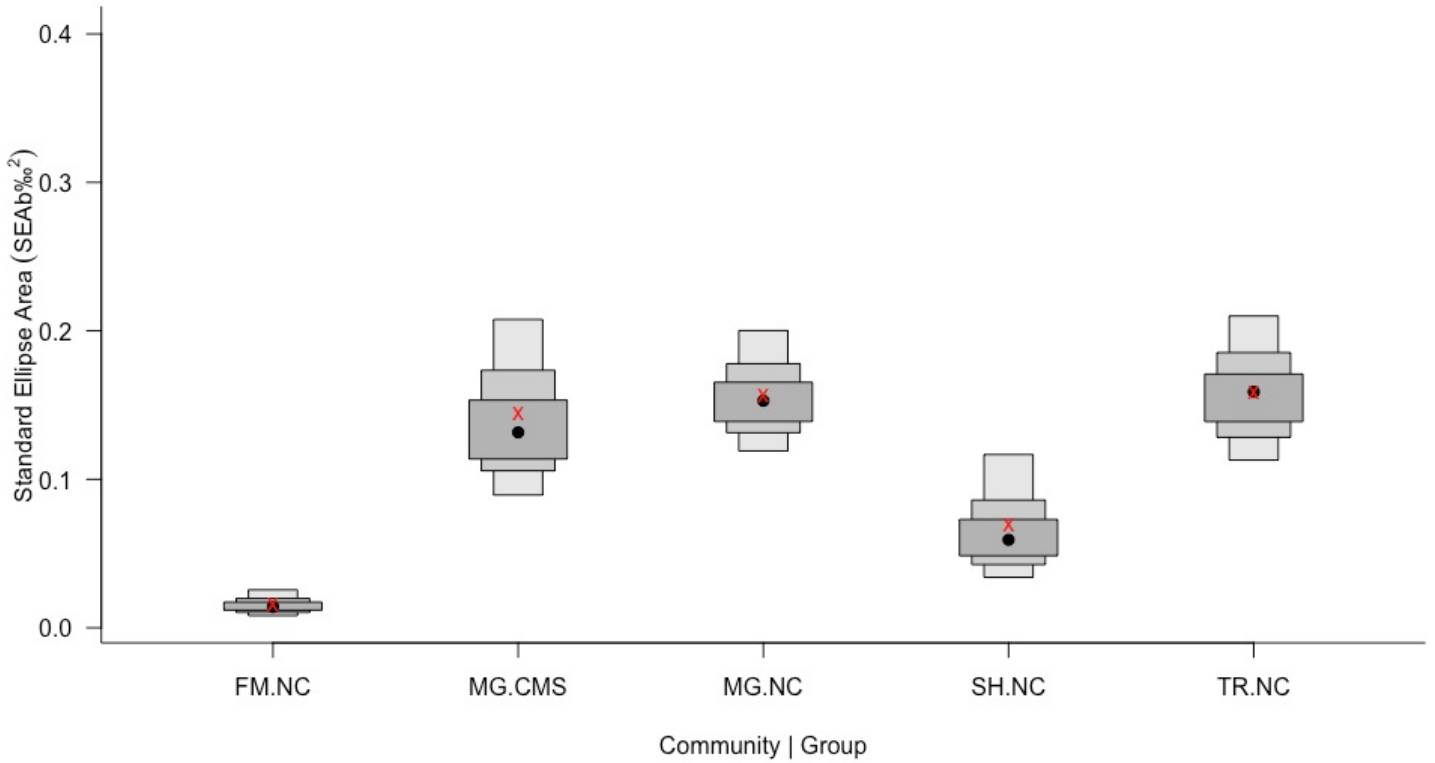


Figure 4.6: SIBER Density plot of isotopic niche widths ($SEA_b\%{}^2$) of sympatric *F. virilis* and *C. chinensis* in McGregor Lake Reservoir and *F. virilis* in the reservoirs where they exist allopatrically. The graduations denote 95, 75, 50% credibility intervals with Bayesian estimates. Black dots represent the bootstrapped mean SEA_b areas and the red crosses represent the small sample size corrected standard ellipse area (SEA_c). Reservoirs: MG= McGregor Lake Reservoir, FM= Forty-Mile Coulee Reservoir, SH= Sherburne Lake Reservoir, SM= St. Mary's Reservoir, TR=Travers Reservoir. Species: NC = Northern Crayfish, *F. virilis*, CMS = Chinese Mystery Snail, *C. chinensis*.

Appendix

Table A4.1: Summary statistics of each species within each reservoir lake. Reservoirs: MG= McGregor Lake Reservoir, FM= Forty-Mile Coulee Reservoir, SH= Sherburne Lake Reservoir, SM= St. Mary's Reservoir, TR=Travers Reservoir. Group: LKWH = Lake Whitefish, NRPK=Northern Pike, WALL= Walleye, WHSC = White sucker, SPSH= Spottail Shiner, YEPE = Yellow perch.

Reservoir	Class	Group	Mean $\delta^{13}C$	St. Dv. $\delta^{13}C$	St. Er. $\delta^{13}C$	Mean $\delta^{15}N$	St. Dv. $\delta^{15}N$	St. Er. $\delta^{15}N$
FM	fish	LKWH	-0.20	0.04	0.01	4.56	0.12	0.03
FM	fish	NRPK	-0.05	0.03	0.01	5.15	0.12	0.03
FM	fish	WALL	-0.04	0.04	0.01	5.34	0.16	0.04
FM	fish	WHSC	-0.03	0.07	0.03	4.28	0.30	0.11
FM	fish	YEPE	-0.03	0.02	0.00	4.48	0.11	0.02
FM	littoral	macrophyte	0.58	0.42	0.11	2.27	0.59	0.15
FM	littoral	Northern crayfish	0.07	0.04	0.01	3.68	0.14	0.04
FM	littoral	parasitic - inverts	-0.02	NA	NA	1.49	NA	NA
FM	littoral	periphyton	1.02	0.22	0.06	1.00	1.09	0.30
FM	littoral	primary consumers	0.00	0.19	0.03	2.00	0.71	0.10
MG	fish	BURB	0.07	0.06	0.02	3.14	0.14	0.05
MG	fish	LKWH	-0.50	0.15	0.04	3.76	0.13	0.03
MG	fish	NRPK	0.02	0.11	0.04	3.45	0.17	0.07
MG	fish	SPSH	-0.44	0.13	0.04	3.04	0.20	0.06
MG	fish	WALL	-0.18	0.25	0.07	4.05	0.15	0.04
MG	littoral	Chinese mystery snail	0.15	0.23	0.05	2.30	0.20	0.04
MG	littoral	macrophyte	0.97	0.89	0.26	1.21	0.35	0.10
MG	littoral	Northern crayfish	0.16	0.26	0.03	2.30	0.25	0.03
MG	littoral	periphyton	1.78	0.67	0.21	0.82	0.50	0.16
MG	littoral	predators - inverts	-0.25	0.54	0.16	2.55	0.36	0.10
MG	littoral	primary consumers	0.00	0.24	0.04	2.00	0.24	0.04
SH	fish	LKWH	-0.28	NA	NA	4.23	NA	NA
SH	fish	NRPK	-0.14	0.06	0.01	4.60	0.17	0.04
SH	fish	SPSH	-0.08	NA	NA	3.75	NA	NA
SH	fish	WALL	-0.24	0.07	0.01	4.71	0.29	0.06
SH	fish	YEPE	-0.23	0.15	0.04	3.95	0.14	0.03
SH	littoral	macrophyte	0.49	0.27	0.07	1.65	0.59	0.15
SH	littoral	Northern crayfish	-0.07	0.08	0.02	2.97	0.28	0.08
SH	littoral	periphyton	0.87	0.22	0.06	1.03	0.46	0.12
SH	littoral	predators - inverts	-0.07	0.14	0.03	2.81	0.69	0.17
SH	littoral	primary consumers	0.00	0.15	0.02	2.00	0.62	0.08
SM	fish	BURB	-0.42	0.03	0.02	3.83	0.12	0.07
SM	fish	LKWH	-0.70	0.13	0.04	3.43	0.30	0.08
SM	fish	LNRC	-0.14	0.12	0.04	2.91	0.29	0.10
SM	fish	NRPK	-0.22	NA	NA	3.84	NA	NA
SM	fish	SPSH	-0.10	0.18	0.03	2.96	0.17	0.03
SM	fish	WALL	-0.17	0.13	0.03	3.88	0.17	0.04
SM	fish	WHSC	-0.02	NA	NA	3.44	NA	NA
SM	littoral	macrophyte	0.64	1.21	0.36	2.51	1.14	0.34
SM	littoral	parasitic - inverts	-0.18	0.14	0.10	2.75	1.49	1.06
SM	littoral	periphyton	1.26	0.46	0.15	1.05	0.78	0.26
SM	littoral	predators - inverts	0.16	0.04	0.03	2.12	0.06	0.05
SM	littoral	primary consumers	0.00	0.27	0.06	2.00	0.21	0.05
TR	fish	BURB	0.18	0.09	0.02	3.25	0.40	0.09
TR	fish	LKWH	-0.26	0.16	0.03	3.81	0.57	0.11
TR	fish	NRPK	0.15	0.14	0.04	3.81	0.44	0.11
TR	fish	SPSH	-0.08	0.05	0.02	3.54	0.17	0.07
TR	fish	WALL	0.08	0.14	0.03	4.37	0.43	0.09
TR	littoral	macrophyte	1.05	0.78	0.23	2.21	0.65	0.19
TR	littoral	Northern crayfish	0.27	0.16	0.02	2.17	0.41	0.06
TR	littoral	periphyton	2.15	0.14	0.07	0.14	0.16	0.08
TR	littoral	predators - inverts	-0.04	0.27	0.09	2.34	1.22	0.41
TR	littoral	primary consumers	0.00	0.29	0.07	2.00	0.63	0.15

Table A4.2: Summary table showing the number of individuals of fish, invasive species, invertebrates, macrophyte and periphyton samples collected at each lake. BURB = Burbot, LKWH = Lake Whitefish, LNSC = Longnose Sucker, NRPK = Northern Pike, WALL = Walleye, WHSC = White sucker, YEPE = Yellow perch, SPSH = Spottail Shiner, CMS = Chinese Mystery Snail, NOCR = Northern Crayfish.

	FM	MG	SH	SM	TR	Grand Total
Treatment	<i>F. virilis</i>	<i>F. virilis</i> & <i>C. chinensis</i>	<i>F. virilis</i>	Control	<i>F. virilis</i>	
Consumers						
BURB		8		3	20	31
LKWH	21	16	1	13	25	76
LNSC				8		8
NRPK	16	6	18	1	15	56
WALL	15	15	25	22	25	102
WHSC	7			1		8
YEPE	21		18			39
SPSH		10	1	38	5	54
Introduced species						
CMS		23				23
NOCR	14	59	12		42	127
Invertebrates (functional - feeding groups)						
collectors - gatherers, shredders, predators	1	16	9		5	31
collectors - gatherers	11	2	4			17
collectors - gatherers, predators, filterers	5	2	4	1	4	16
filtering collectors			1			1
generally shredders - herbivores					2	2
native clams	25		4			29
nativesnail_baseline1	3	1	24	11		39
parasitic	1			2		3
piercers - herbivores, predators, or scrapers		2	9		5	16
predators		6	5		1	12
predators - engulfers		5	6		3	14
predators - piercers			5	1	3	9
predators - scavengers		1		1	2	4
chironomid_baseline2	3	15	9	7	2	36
Macrophytes and periphyton						
macrophyte	15	12	15	11	12	65
periphyton	13	10	14	9	4	50
Grand Total	171	209	184	129	175	868

Table A4.3: The relative contribution of putative resources to the diet of Lake Whitefish (LKWH), Northern Pike (NRPK), and Walleye (WALL).

McGregor Lake Reservoir	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Epsilon.1	3.249	1.735	0.847	1.076	1.965	2.926	4.169	6.529	7.481
Epsilon.2	0.429	0.144	0.227	0.251	0.33	0.404	0.505	0.697	0.767
BRB.Chinese mystery snail	0.056	0.056	0.001	0.003	0.016	0.039	0.079	0.168	0.211
BRB.macrophyte	0.076	0.078	0.002	0.004	0.019	0.049	0.106	0.244	0.296
BRB.Northern crayfish	0.055	0.054	0.002	0.003	0.016	0.038	0.076	0.161	0.2
BRB.periphyton	0.044	0.046	0.001	0.002	0.011	0.028	0.062	0.141	0.17
BRB.primary consumers	0.456	0.159	0.083	0.138	0.361	0.484	0.575	0.67	0.693
BRB.SPSH	0.313	0.08	0.187	0.201	0.257	0.302	0.361	0.465	0.496
LKWH.Chinese mystery snail	0.014	0.018	0	0	0.003	0.008	0.018	0.05	0.067
LKWH.macrophyte	0.022	0.03	0	0.001	0.004	0.01	0.027	0.09	0.115
LKWH.Northern crayfish	0.014	0.017	0	0	0.003	0.008	0.019	0.048	0.06
LKWH.periphyton	0.007	0.008	0	0	0.002	0.005	0.01	0.025	0.03
LKWH.primary consumers	0.105	0.065	0.008	0.013	0.051	0.098	0.15	0.222	0.246
LKWH.SPSH	0.837	0.062	0.712	0.731	0.794	0.838	0.883	0.937	0.948
NRPK.Chinese mystery snail	0.06	0.081	0	0.001	0.009	0.029	0.077	0.231	0.293
NRPK.macrophyte	0.042	0.044	0.001	0.002	0.01	0.027	0.059	0.132	0.169
NRPK.Northern crayfish	0.057	0.075	0.001	0.001	0.009	0.028	0.074	0.209	0.282
NRPK.periphyton	0.029	0.033	0	0.001	0.006	0.017	0.041	0.097	0.115
NRPK.primary consumers	0.32	0.135	0.037	0.077	0.228	0.329	0.42	0.522	0.554
NRPK.SPSH	0.493	0.09	0.32	0.356	0.433	0.487	0.551	0.645	0.683
WALL.Chinese mystery snail	0.013	0.016	0	0	0.002	0.007	0.017	0.045	0.058
WALL.macrophyte	0.009	0.009	0	0	0.002	0.006	0.013	0.029	0.034
WALL.Northern crayfish	0.012	0.016	0	0	0.002	0.006	0.016	0.045	0.058
WALL.periphyton	0.006	0.007	0	0	0.002	0.004	0.008	0.02	0.025
WALL.primary consumers	0.036	0.024	0.004	0.006	0.018	0.031	0.049	0.084	0.098
WALL.SPSH	0.924	0.035	0.843	0.86	0.902	0.929	0.95	0.972	0.977
St. Mary's Reservoir	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Epsilon.1	0.115	0.064	0.034	0.041	0.069	0.101	0.144	0.23	0.271
Epsilon.2	0.196	0.079	0.078	0.091	0.139	0.186	0.237	0.34	0.388
BRB.macrophyte	0.53	0.105	0.331	0.357	0.442	0.513	0.59	0.702	0.735
BRB.periphyton	0.016	0.018	0	0.001	0.004	0.01	0.021	0.053	0.066
BRB.primary consumers	0.05	0.049	0.001	0.002	0.014	0.034	0.07	0.153	0.182
BRB.SPSH	0.414	0.111	0.188	0.226	0.339	0.419	0.494	0.589	0.616
LKWH.macrophyte	0.81	0.103	0.59	0.624	0.741	0.821	0.891	0.958	0.971
LKWH.periphyton	0.014	0.021	0	0	0.002	0.006	0.018	0.058	0.077
LKWH.primary consumers	0.056	0.07	0	0.001	0.006	0.024	0.083	0.208	0.244
LKWH.SPSH	0.12	0.08	0.015	0.02	0.058	0.104	0.168	0.273	0.317
NRPK.macrophyte	0.409	0.167	0.173	0.192	0.286	0.377	0.496	0.751	0.822
NRPK.periphyton	0.017	0.026	0	0	0.002	0.007	0.02	0.068	0.091
NRPK.primary consumers	0.05	0.065	0.001	0.001	0.008	0.025	0.066	0.185	0.243
NRPK.SPSH	0.524	0.183	0.096	0.159	0.412	0.559	0.659	0.772	0.802
WALL.macrophyte	0.312	0.057	0.206	0.22	0.272	0.311	0.35	0.407	0.427
WALL.periphyton	0.007	0.009	0	0	0.002	0.004	0.01	0.025	0.032
WALL.primary consumers	0.018	0.019	0	0.001	0.005	0.012	0.025	0.056	0.071
WALL.SPSH	0.663	0.06	0.541	0.562	0.624	0.665	0.703	0.757	0.776
Travers Lake Reservoir	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Epsilon.1	2.031	0.984	0.624	0.711	1.261	1.908	2.615	3.875	4.36
Epsilon.2	2.211	0.56	1.284	1.414	1.825	2.147	2.532	3.23	3.514
BRB.macrophyte	0.019	0.021	0	0.001	0.005	0.012	0.027	0.061	0.074
BRB.Northern crayfish	0.238	0.135	0.006	0.017	0.127	0.249	0.346	0.444	0.469
BRB.periphyton	0.035	0.031	0.001	0.001	0.008	0.025	0.056	0.095	0.103
BRB.primary consumers	0.41	0.088	0.245	0.273	0.347	0.409	0.472	0.556	0.58
BRB.SPSH	0.298	0.077	0.158	0.176	0.243	0.296	0.35	0.43	0.46
LKWH.macrophyte	0.004	0.005	0	0	0.001	0.002	0.006	0.015	0.019
LKWH.Northern crayfish	0.025	0.021	0.001	0.002	0.009	0.02	0.034	0.064	0.079
LKWH.periphyton	0.004	0.003	0	0	0.001	0.003	0.005	0.011	0.013
LKWH.primary consumers	0.718	0.129	0.496	0.518	0.62	0.706	0.818	0.94	0.958
LKWH.SPSH	0.249	0.128	0.02	0.034	0.148	0.258	0.346	0.449	0.476
NRPK.macrophyte	0.017	0.022	0	0	0.003	0.008	0.023	0.064	0.083
NRPK.Northern crayfish	0.154	0.099	0.004	0.01	0.069	0.149	0.226	0.321	0.354
NRPK.periphyton	0.021	0.019	0	0.001	0.005	0.015	0.033	0.06	0.068
NRPK.primary consumers	0.306	0.093	0.133	0.158	0.244	0.302	0.366	0.461	0.493
NRPK.SPSH	0.502	0.097	0.311	0.341	0.441	0.505	0.567	0.654	0.68
WALL.macrophyte	0.006	0.009	0	0	0.001	0.003	0.008	0.022	0.029
WALL.Northern crayfish	0.036	0.028	0.001	0.003	0.014	0.029	0.051	0.091	0.106
WALL.periphyton	0.006	0.006	0	0	0.002	0.004	0.008	0.018	0.021
WALL.primary consumers	0.106	0.063	0.016	0.022	0.058	0.096	0.141	0.221	0.25
WALL.SPSH	0.846	0.067	0.698	0.726	0.805	0.853	0.897	0.943	0.954
Shoaburg Reservoir	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Epsilon.1	0.387	0.159	0.156	0.18	0.273	0.36	0.47	0.682	0.76
Epsilon.2	0.883	0.221	0.545	0.581	0.719	0.852	1.006	1.277	1.397
LKWH.macrophyte	0.03	0.03	0.001	0.001	0.009	0.021	0.042	0.09	0.109
LKWH.Northern crayfish	0.102	0.077	0.007	0.012	0.044	0.083	0.139	0.253	0.299
LKWH.periphyton	0.019	0.019	0.001	0.001	0.006	0.013	0.027	0.058	0.071
LKWH.primary consumers	0.075	0.06	0.003	0.006	0.028	0.061	0.106	0.199	0.224
LKWH.YEPE	0.774	0.078	0.604	0.636	0.724	0.781	0.83	0.886	0.905
NRPK.macrophyte	0.029	0.03	0	0.001	0.006	0.018	0.044	0.093	0.103
NRPK.Northern crayfish	0.148	0.103	0.004	0.008	0.059	0.139	0.224	0.325	0.352
NRPK.periphyton	0.018	0.02	0	0.001	0.003	0.011	0.027	0.062	0.074
NRPK.primary consumers	0.052	0.043	0.002	0.003	0.018	0.042	0.076	0.138	0.156
NRPK.YEPE	0.752	0.068	0.614	0.635	0.705	0.755	0.805	0.855	0.868
WALL.macrophyte	0.01	0.011	0	0	0.002	0.006	0.013	0.032	0.04
WALL.Northern crayfish	0.035	0.033	0.001	0.002	0.01	0.025	0.05	0.107	0.124
WALL.periphyton	0.006	0.007	0	0	0.001	0.004	0.008	0.02	0.025
WALL.primary consumers	0.033	0.028	0.001	0.001	0.01	0.026	0.051	0.088	0.099
WALL.YEPE	0.916	0.035	0.84	0.854	0.895	0.919	0.942	0.969	0.976
Forty Mile Reservoir	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Epsilon.1	13.465	3.095	7.932	8.603	11.144	13.32	15.836	18.76	19.329
Epsilon.2	0.221	0.05	0.143	0.152	0.185	0.215	0.25	0.314	0.334
LKWH.macrophyte	0.011	0.019	0	0	0.002	0.006	0.013	0.035	0.048
LKWH.Northern crayfish	0.055	0.052	0.001	0.003	0.017	0.04	0.078	0.163	0.195
LKWH.periphyton	0.005	0.006	0	0	0.001	0.003	0.007	0.018	0.022
LKWH.primary consumers	0.337	0.032	0.276	0.289	0.321	0.339	0.357	0.383	0.39
LKWH.YEPE	0.591	0.042	0.487	0.509	0.57	0.599	0.621	0.645	0.652
NRPK.macrophyte	0.005	0.007	0	0	0.001	0.002	0.006	0.018	0.025
NRPK.Northern crayfish	0.031	0.038	0	0.001	0.006	0.017	0.043	0.11	0.141
NRPK.periphyton	0.003	0.004	0	0	0	0.001	0.003	0.01	0.013
NRPK.primary consumers	0.115	0.021	0.071	0.079	0.103	0.117	0.13	0.146	0.152
NRPK.YEPE	0.846	0.031	0.764	0.789	0.832	0.852	0.866	0.885	0.892
WALL.macrophyte	0.003	0.005	0	0	0	0.001	0.004	0.012	0.017
WALL.Northern crayfish	0.017	0.021	0	0.001	0.003	0.009	0.023	0.063	0.08
WALL.periphyton	0.002	0.003	0	0	0	0.001	0.002	0.007	0.009
WALL.primary consumers	0.046	0.016	0.016	0.019	0.035	0.046	0.057	0.074	0.079
WALL.YEPE	0.932	0.023	0.878	0.891	0.919	0.934	0.947	0.963	0.969

Chapter 5

General Conclusions

Once invasive species are established, they can have complex impacts on the ecosystems and regions and can often have impacts on local economies (Rennie et al. 2009). This thesis aimed to describe the implications involved with the novel introduction of Chinese Mystery Snail, *Cipangopaludina chinensis*, in Alberta, Canada and their interactions with Northern Crayfish, *F. virilis*, another invasive species sharing the same waterbody. As invasive species continue to radiate from points of introduction, it is critical to understand how these species will impact their new ecosystems. Without this imperative knowledge it is very difficult for managers and conservationists to effectively manage these species, if mitigating the introduction fails. Research provides insight to management methods and can help justifying allocating a certain amount of funds towards a project.

My hopes are that my findings will help advise management programs related to both *C. chinensis* and *F. virilis* in Alberta lakes and reservoir systems, and provide important information regarding the trophic interactions and outcomes to native recreational fish species in Alberta's lakes. In Chapter 2, I synthesized information in comprehensive literature review on *C. chinensis* to determine what makes them particularly prone to establish waterbodies where they are introduced and what could make this species volatile or impactful to aquatic ecosystems. Then, with the contributions of other experts in the field, growth-rate analysis, DNA barcoding, and investigation into whether the *C. chinensis* individuals carry parasitic cercariae was conducted (Chapter 3). In Chapter 4, I used stable isotope analysis and gut-content analysis to investigate

the different community and group effects that both *C. chinensis* and *F. virilis* might have on native fish species.

Throughout Chapter 3, we documented the initial finding of *C. chinensis* in Alberta and biological information that will aid management efforts. We confirmed specimens as *C. chinensis* (Gray, 1834) through DNA barcoding and added the barcode to GenBank. We also conducted analysis of growth rate, fecundity, and assessed whether Albertan *C. chinensis* species are infected by digenean trematodes. We discovered, after a 24-hour experimental period, that *C. chinensis* specimens that are currently in Alberta are not infected by digenean trematodes, as no cercariae were present. We also determined that shell growth quickly outpaces growth in shell width, and that upon emergence *C. chinensis* are larger than many of the native snail species. Annual fecundity of Alberta *C. chinensis* produce offspring at lower average juveniles per female (19.3 juveniles) compared to average annual fecundity of populations elsewhere (25-30 juveniles per female), however even one gravid female is sufficient to found a population (Stephen et al. 2013).. We provided recommendations to where searches for *C. chinensis* should be focused, notably near artificial substrates, riprap, and submerged vegetation, where there is stagnant water (Solomon et al. 2010; Chaine 2012). We suggested that *C. chinensis* met the criteria for four of the five stages of species invasion: arrival, establishment, growth and reproduction, however there was no evidence for the fifth stage, dispersal (Lockwood et al. 2007), of *C. chinensis* from McGregor Lake Reservoir other waterbodies

In Chapter 4, I addressed the trophic consequences that may arise with the addition of *F. virilis* and *C. chinensis* to aquatic ecosystems in southern Alberta, and their impacts to three recreational fish species: Lake Whitefish (*Coregonus clupeaformis*), Northern Pike (*Esox lucius*), and Walleye (*Sander vitreus*). Specifically, my main objectives of this study were to 1) determine the difference in trophic characteristics between reservoir communities where invasive

species are sympatric, where only invasive crayfish are present, and where there are no invasive species present, and; 2) describe the trophic interaction between *C. chinensis* and *F. virilis* in aquatic food webs. I hypothesized that the presence of only one aquatic invader would create bottom-up effects on the food-web, and an additional invasive species would amplify these effects, creating shifts in fish trophic positions by moving basal energy away from the higher trophic levels through consumption of algae and macrophytes, or by providing a new prey resource (Twardochleb and Olden 2016; Kingsbury et al. 2021). I also hypothesized that *C. chinensis* and *F. virilis* would have overlapping niches, either through competition for resources or through the predation of *C. chinensis* by *F. virilis* (Johnson et al. 2009). I used stable isotope analysis and gut-content analysis, jointly, to help determine answers and address these objectives. I established that fish are becoming more piscivorous compared to reference lakes where there are no invasive species. Especially Lake Whitefish which is switching from macrophytes to a diet comprised mainly of secondary consumers, such as Spottail Shiner, a behaviour that happens when they are searching for nutrients or are experiencing poor body condition (Brownscombe and Fox 2013). That said, there may be an “adaptive lag” period prior to fish preying on either of the invasives outlined in this study (Carlsson et al. 2009). Reservoir ecosystems where only *F. virilis* was present has narrower carbon ranges than those that had both invasive species, which may mean that locations with both invasive species have more niche diversification than those with just one invasive species, *F. virilis*. Changes in average fish trophic position were significantly higher with the addition of only *F. virilis* into a waterbody, but with the addition of *C. chinensis* resembled more the reservoir without invasive species present. This could be happening as a result of the fish diet switching and diversifying their diets for nutrients, they require but cannot acquire from macrophytes that are lacking from their diets (Pothoven and Nalepa 2006). Macrophytes may be lacking due, simply, to the presence of either

C. chinensis and *F. virilis*, both species known to decimate macrophytes in invaded waterbodies (Carpenter and Lodge 1986; Lewis et al. 2001; Cross and Benke 2002; Johnson et al. 2009; Sura and Mahon 2011; Kingsbury et al. 2021). I also discovered that the trophic niche ellipses of *C. chinensis* and *F. virilis* significantly overlap, which points to competitive exclusion (Meszéna et al. 2006). Since the total area of *F. virilis* is larger than that of *C. chinensis*, crayfish may eventually outcompete *C. chinensis*.

My research addressed my overall goal to investigate the novel introduction of *C. chinensis* and the potential impacts of *C. chinensis* and *F. virilis* on native recreational fish species in reservoir ecosystems in southern Alberta. The findings gained throughout these studies are useful for the management of *C. chinensis* and *F. virilis* in southern Alberta and will hopefully provide a catalyst for other researchers and resource managers.

Literature Referenced

- Alberta Environment and Parks (AEP). (n.d.). Chinese Mystery Snail (*Cipangopaludina chinensis*) - Open Government. <https://open.alberta.ca/publications/chinese-mystery-snail-alert#detailed>. Accessed 01-01-2021.
- Amundsen, P.-A., and J. Sánchez-Hernández. 2019. Feeding studies take guts – critical review and recommendations of methods for stomach contents analysis in fish. *Journal of Fish Biology* 95(6):1364–1373.
- Anderson, C., and G. Cabana. 2007. Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. *Journal of the North American Benthological Society* 26(2):273–285.
- Ballari, S. A., S. E. Kuebbing, and M. A. Nuñez. 2016. Potential problems of removing one invasive species at a time: a meta-analysis of the interactions between invasive vertebrates and unexpected effects of removal programs. *PeerJ* 4:e2029.
- Benson, A. (n.d.). 1 Native Range and Status in the United States:16.
- Bhushan B. Biomimetics: Lessons from nature—an overview. *Philosophical Transactions*, 2009, 367, 1445–1486
- Bond, A. L., and A. W. Diamond. 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecological Applications* 21(4):1017–1023.
- Brownscombe JW, Fox MG (2013) Living at the edge of the front; reduced predation risk to invasive round goby in a Great Lakes tributary. *Hydrobiologia* 707:199–208.
<https://doi.org/10.1007/s10750-012-1427-z>
- Burch, J., And B. Jb. 1980. A Guide To The Freshwater Snails Of The Philippines. A Guide To The Freshwater Snails Of The Philippines.

- Burnett, J. L., K. Pope L., A. Wong, C. R. Allen, D. M. Haak, B. J. Stephen, and D. R. Uden. 2018. Thermal Tolerance Limits of the Chinese Mystery Snail (*Bellamya chinensis*): Implications for Management. *American Malacological Bulletin* 36(1):140–144.
- Bury, J. A., B. E. Sietman, and B. N. Karns. 2007. Distribution of the Non-Native Viviparid Snails, *Bellamya chinensis* and *Viviparus georgianus*, in Minnesota and the First Record of *Bellamya japonica* from Wisconsin. *Journal of Freshwater Ecology* 22(4):697–703.
- Carlsson NO, Sarnelle O, Strayer DL (2009) Native predators and exotic prey –an acquired taste? *Frontiers in Ecology and the Environment* 7:525–532.
<https://doi.org/10.1890/080093>
- Carpenter SR, Lodge DM (1986) Effects of submersed macrophytes on ecosystem processes. *Aquatic Botany* 26:341–370. [https://doi.org/10.1016/0304-3770\(86\)90031-8](https://doi.org/10.1016/0304-3770(86)90031-8)
- Carpenter SR, Stanley EH, Vander Zanden MJ (2011) State of the World’s Freshwater Ecosystems: Physical, Chemical, and Biological Changes. *Annu Rev Environ Resour* 36:75–99. <https://doi.org/10.1146/annurev-environ-021810-094524>
- Carpenter, S. R., and D. M. Lodge. 1986. Effects of submersed macrophytes on ecosystem processes. *Aquatic Botany* 26:341–370.
- Chaine N (2012) Population estimate of Chinese Mystery Snail (*Bellamya chinensis*) in a Nebraska reservoir. *BIR* 1:283–287. <https://doi.org/10.3391/bir.2012.1.4.07>
- Chaine, N. 2012. Population estimate of Chinese Mystery Snail (*Bellamya chinensis*) in a Nebraska reservoir. *BioInvasions Records* 1(4):283–287.
- Chiu, Y.-W., H.-C. Chen, S.-C. Lee, and C. A. Chen. 2002. Morphometric Analysis of Shell and Operculum Variations in the Viviparid Snail, *Cipangopaludina chinensis* (Mollusca: Gastropoda), in Taiwan. *Zoological Studies*:11.

- Chung, P. R., and Y. Jung. 1999. *Cipangopaludina chinensis malleata* (Gastropoda: Viviparidae): A New Second Molluscan Intermediate Host of a Human Intestinal Fluke *Echinostoma cinetorchis* (Trematoda: Echinostomatidae) in Korea. *The Journal of Parasitology* 85(5):963–964. [The American Society of Parasitologists, Allen Press].
- Clarke, A. H. 1981. *The freshwater molluscs of Canada*. Ottawa : National Museum of Natural Sciences, National Museums of Canada.
- Clifford HF (1991) *Aquatic Invertebrates of Alberta*. University of Alberta
- Collas, F., S. Breedveld, J. Matthews, G. van der Velde, and R. Leuven. 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.
- Cowie, R. H. (n.d.). Patterns of introduction of non-indigenous non-marine snails and slugs in the Hawaiian Islands:20.
- Cross WF, Benke AC (2002) Intra- and interspecific competition among coexisting lotic snails. *Oikos* 96:251–264. <https://doi.org/10.1034/j.1600-0706.2002.960207.x>
- Cross, W. F., and A. C. Benke. 2002. Intra- and interspecific competition among coexisting lotic snails. *Oikos* 96(2):251–264.
- David, A., and S. Cote. 2019. Genetic evidence confirms the presence of the Japanese mystery snail, *Cipangopaludina japonica* (von Martens, 1861) (Caenogastropoda: Viviparidae) in northern New York. *BioInvasions Records* 8(4):793–803.
- Diana, J. S. 1979. The feeding pattern and daily ration of a top carnivore, the Northern Pike (*Esox lucius*). *Canadian Journal of Zoology* 57(11):2121–2127.

- Distler, D. A. 2003. Occurrence of the mystery snail *Cipangopaludina chinensis* (Gastropoda: Viviparidae) in the Walnut River basin, Kansas. Transactions of the Kansas Academy of Science.
- Doll, J. C., and S. J. Jacquemin. 2021. Incorporation of feeding functional group information informs explanatory patterns of long-term population changes in fish assemblages. PeerJ 9:e11032.
- Dorn, N. J. 2013. Consumptive effects of crayfish limit snail populations. Freshwater Science 32(4):1298–1308.
- Drake, D. A. R., and N. E. Mandrak. 2014. Bycatch, bait, anglers, and roads: quantifying vector activity and propagule introduction risk across lake ecosystems. Ecological Applications 24(4):877–894.
- Edgar, M. R., P. C. Hanington, R. Lu, H. Proctor, R. Zurawell, N. Kimmel, and M. S. Poesch. 2022. The first documented occurrence and life history characteristics of the Chinese Mystery Snail, *Cipangopaludina chinensis* (Gray, 1834) (Mollusca: Viviparidae), in Alberta, Canada. BioInvasions Records:12.
- Edwards, M. S., T. F. Turner, and Z. D. Sharp. 2002. Short- and Long-Term Effects of Fixation and Preservation on Stable Isotope Values ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$) of Fluid-Preserved Museum Specimens. Copeia 2002(4):1106–1112.
- Firth BL, Poesch MS, Koops MA, et al (2021) Diet overlap of common and at-risk riverine benthic fishes before and after Round Goby (*Neogobius melanostomus*) invasion. Biol Invasions 23:221–234. <https://doi.org/10.1007/s10530-020-02366-7>
- Gangloff, M. M., K. K. Lenertz, and J. W. Feminella. 2008. Parasitic mite and trematode abundance are associated with reduced reproductive output and physiological condition of freshwater mussels. Hydrobiologia 610(1):25.

GBIF.org. (n.d.).GBIF Occurrence Download. <https://doi.org/10.15468/dl.qthvrh>. Accessed 13-01-2021.

Gelman, A., and D. B. Rubin. 1992. Inference from Iterative Simulation Using Multiple Sequences. *Statistical Science* 7(4):457–472.

GISD (Global Invasive Species Database). 2017. *Bellamya chinensis* (mollusc). <http://www.issg.org/-database/species/ecology.asp?si=1812&fr=1&sts=sss&lang=EN>. Accessed 01-01-2021.

Gong, Y., J. Lu, X. Wang, and Y. Huang. (n.d.). Response of Hatchery-Cultured Mud Crab (*Scylla paramamosain*) Instar Fed Natural Prey and an Artificial Feed:7.

Government of Alberta. 2012, September 17. Alberta Queen's Printer: Text. https://www.qp.alberta.ca/1266.cfm?page=F16.cfm&leg_type=Acts&isbncln=9780779790432&display=html. Accessed 01-01-2021.

Government of British Columbia. (n.d.). Invasive Species Alert! Chinese Mystery (*Cipangopaludina chinensis*). https://www2.gov.bc.ca/assets/gov/environment/plants-animals-and-ecosystems/invasive-species/alerts/chinese_mystery_snail_alert.pdf. Accessed 01-01-2021.

Government of Manitoba. The Water Protection Act 2015. https://web2.gov.mb.ca/laws/regs/current/_pdf-regs.php?reg=173/2015 Accessed 15-01-2021.

Haak, D. M. (n.d.). BIOENERGETICS AND HABITAT SUITABILITY MODELS FOR THE CHINESE MYSTERY SNAIL (*BELLAMYA CHINENSIS*):249.

Haak, D. M. 2015. bioenergetics and habitat suitability models for the Chinese Mystery Snail *Bellamya chinensis*. null.

- Haak, D. M., B. J. Stephen, R. A. Kill, N. A. Smeenk, C. R. Allen, and K. L. Pope. 2014. Toxicity of copper sulfate and rotenone to Chinese Mystery Snail (*Bellamya chinensis*). Management of Biological Invasions.
- Haak, D. M., N. M. Chaine, B. J. Stephen, A. Wong, and C. R. Allen. 2013. mortality estimate of Chinese Mystery Snail *Bellamya chinensis* reeve 1863 in a Nebraska reservoir. BioInvasions Records.
- Harried, B., K. Fischer, K. Perez, and G. Sandland. 2015. Assessing infection patterns in Chinese Mystery Snails from Wisconsin, USA using field and laboratory approaches. Aquatic Invasions 10(2):169–175.
- Havel JE, Kovalenko KE, Thomaz SM, et al (2015) Aquatic invasive species: challenges for the future. Hydrobiologia 750:147–170. <https://doi.org/10.1007/s10750-014-2166-0>
- 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.
- Havel, J. E., L. A. Bruckerhoff, M. A. Funkhouser, and A. R. Gemberling. 2014. Resistance to desiccation in aquatic invasive snails and implications for their overland dispersal. Hydrobiologia 741(1):89–100.
- Hirano, T., T. Saito, Y. Tsunamoto, J. Koseki, B. Ye, V. T. Do, O. Miura, Y. Suyama, and S. Chiba. 2019. Enigmatic incongruence between mtDNA and nDNA revealed by multi-locus phylogenomic analyses in freshwater snails. Scientific Reports 9(1):6223.
- Ho, J.-Z., P.-H. Chiang, C.-H. Wu, and P.-S. Yang. 2010. Life cycle of the aquatic firefly *Luciola ficta* (Coleoptera: Lampyridae). Journal of Asia-Pacific Entomology 13(3):189–196.

- Hoeinghaus, D. J., and S. C. Zeug. 2008. Can Stable Isotope Ratios Provide for Community-Wide Measures of Trophic Structure? Comment. *Ecology* 89(8):2353–2357.
- Hoffman, J. C., and T. T. Sutton. 2010. Lipid correction for carbon stable isotope analysis of deep-sea fishes. *Deep Sea Research Part I: Oceanographic Research Papers* 57(8):956–964.
- ITIS Results of Search in every Kingdom for Scientific Name containing “*Cipangopaludina chinensis*.” (n.d.). . <https://www.itis.gov/servlet/SingleRpt/SingleRpt>. Accessed 07-01-2020.
- Jackson, A. L., R. Inger, A. C. Parnell, and S. Bearhop. 2011. Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80(3):595–602.
- Johnson PTJ, Olden JD, Solomon CT, Vander Zanden MJ (2009) Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia* 159:161–170. <https://doi.org/10.1007/s00442-008-1176-x>
- Johnson, P. T. J., J. D. Olden, C. T. Solomon, and M. J. Vander Zanden. 2009. Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia* 159(1):161–170.
- Jokinen EH (1992) The freshwater snails (Mollusca: Gastropoda) of New York State. New York State Museum bulletin (USA)
- Jokinen, E. H. 1992. The freshwater snails (Mollusca: Gastropoda) of New York State. New York State Museum bulletin (USA). University of the State of New York, State Education Dept., New York State Museum, Biological Survey.
- Jokinen, E.J. 1982. *Cipangopaludina chinensis* (Gastropoda: Viviparidae) in North America, 950 Review and Update. *Nautilus*. (n.d.). .

- Kim, G., H. Kim, and M.-J. Song. 2018. Ethnopharmacological implications of quantitative and network analysis for traditional knowledge regarding the medicinal use of animals by indigenous people in Wolchulsan National Park, Korea. *Journal of Ethnopharmacology* 213:1–11.
- Kingsbury S, Fong M, McAlpine DF, Campbell L (2021) Assessing the probable distribution of the potentially invasive Chinese Mystery Snail, *Cipangopaludina chinensis*, in Nova Scotia using a random forest model approach. *Aquatic Invasions* 19
- Kingsbury, S. E., D. F. McAlpine, Y. Cheng, E. Parker, and L. M. Campbell. 2020. A review of the non-indigenous Chinese Mystery Snail, *Cipangopaludina chinensis* (Viviparidae), in North America, with emphasis on occurrence in Canada and the potential impact on indigenous aquatic species. *Environmental Reviews:er-2020-0064*.
- Kingsbury, S. E., D. F. McAlpine, Y. Cheng, E. Parker, and L. M. Campbell. 2020. A review of the non-indigenous Chinese Mystery Snail, *Cipangopaludina chinensis* (Viviparidae), in North America, with emphasis on occurrence in Canada and the potential impact on indigenous aquatic species. *Environmental Reviews:er-2020-0064*.
- Kingsbury, S., M. Fong, D. F. McAlpine, and L. Campbell. 2021. Assessing the probable distribution of the potentially invasive Chinese Mystery Snail, *Cipangopaludina chinensis*, in Nova Scotia using a random forest model approach. *Aquatic Invasions:19*.
- Kurihara, Y., and T. Suzuki. 1987. Removal of Heavy Metals and Sewage Sludge Using the Mud Snail, *Cipangopaludina chinensis* malle at a REEVE, in Paddy Fields as Artificial Wetlands. *Water Science and Technology* 19(12):281–286.
- Layman, C. A., D. A. Arrington, C. G. Montaña, and D. M. Post. 2007. Can Stable Isotope Ratios Provide for Community-Wide Measures of Trophic Structure? *Ecology* 88(1):42–48.

- Layman, C. A., M. S. Araujo, R. Boucek, C. M. Hammerschlag-Peyer, E. Harrison, Z. R. Jud, P. Matich, A. E. Rosenblatt, J. J. Vaudo, L. A. Yeager, D. M. Post, and S. Bearhop. 2012. Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews* 87(3):545–562.
- Lee, K. B., J. S. Kim, S. T. Kwak, W. Sim, J. H. Kwak, and Y. S. Kim. 1998. Isolation and identification of chondroitin sulfates from the mud snail. *Archives of Pharmacal Research* 21(5):555–558.
- Levine, J. M., and C. M. D’Antonio. 2003. Forecasting Biological Invasions with Increasing International Trade. *Conservation Biology* 17(1):322–326.
- Lewis WM, Hamilton SK, Rodríguez MA, et al (2001) Foodweb analysis of the Orinoco floodplain based on production estimates and stable isotope data. *Journal of the North American Benthological Society* 20:241–254. <https://doi.org/10.2307/1468319>
- Lewis, W. M., S. K. Hamilton, M. A. Rodríguez, J. F. Saunders, and M. A. Lasi. 2001. Foodweb analysis of the Orinoco floodplain based on production estimates and stable isotope data. *Journal of the North American Benthological Society* 20(2):241–254.
- Li, K. 2012. Molecular analysis of intestinal bacterial communities in *Cipangopaludina chinensis* used in aquatic ecological restorations. *Ecological Engineering* 39:36–39.
- Loffredo, J. R., B. C. Moore, B. Lanouette, R. W. Lee, S. L. Katz, and B. K. Cross. 2019. Trophic status of a non-native crayfish in an oligotrophic lake: bottom-up view of a mixed warmwater and coldwater sport fishery food web. *Lake and Reservoir Management* 35(4):396–414.
- Luo, J., Y. Ye, Z. Gao, Y. Wang, and W. Wang. 2016. Trace Element (Pb, Cd, and As) Contamination in the Sediments and Organisms in Zhalong Wetland, Northeastern

China. *Soil and Sediment Contamination: An International Journal* 25(4):395–407.
Taylor & Francis.

Matthews, J., F. P. L. Collas, L. de Hoop, G. van der Velde, and R. S. E. W. Leuven. 2017.
Risk assessment of the alien Chinese Mystery Snail (*Bellamya chinensis*).
Environmental Sciences.

McAlpine, D., D. Lepitzki, F. Schueler, F. McAlpine, A. Hebda, R. Forsyth, A. Nicolai, J.
Maunder, and R. Noseworthy. 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.

McAlpine, D., D. Lepitzki, F. Schueler, F. McAlpine, A. Hebda, R. Forsyth, A. Nicolai, J.
Maunder, and R. Noseworthy. 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.

Meszéna G, Gyllenberg M, Pásztor L, Metz JAJ (2006) Competitive exclusion and limiting
similarity: A unified theory. *Theoretical Population Biology* 69:68–87.
<https://doi.org/10.1016/j.tpb.2005.07.001>

Micheli, F., and B. S. Halpern. 2005. Low functional redundancy in coastal marine
assemblages. *Ecology Letters* 8(4):391–400.

Mitchell, P., E. E. Prepas, and J. M. Crosby. 1990. *Atlas of Alberta Lakes*. University of
Alberta Press.

- Mo, S., X. Zhang, Y. Tang, Z. Liu, and N. Kettridge. 2017. Effects of snails, submerged plants and their coexistence on eutrophication in aquatic ecosystems. *Knowledge & Management of Aquatic Ecosystems* (418):44.
- Momot, W. T. 1984. Crayfish Production: A Reflection of Community Energetics. *Journal of Crustacean Biology* 4(1):35–54.
- Neucker, T. V. den, T. Schildermans, and K. Scheers. 2017. The invasive Chinese Mystery Snail *Bellamya chinensis* (Gastropoda: Viviparidae) expands its European range to Belgium. *Knowledge and Management of Aquatic Ecosystems*.
- Nicacio G, Juen L (2015) Chironomids as indicators in freshwater ecosystems: an assessment of the literature. *Insect Conservation and Diversity* 8:393–403.
<https://doi.org/10.1111/icad.12123>
- Nilsson, E., C. T. Solomon, K. A. Wilson, T. V. Willis, B. Larget, and M. J. V. Zanden. 2012. Effects of an invasive crayfish on trophic relationships in north-temperate lake food webs. *Freshwater Biology* 57(1):10–23.
- O’Dowd DJ, Green PT, Lake PS (2003) Invasional ‘meltdown’ on an oceanic island. *Ecology Letters* 6:812–817. <https://doi.org/10.1046/j.1461-0248.2003.00512.x>
- O’Reilly, C. M., R. E. Hecky, A. S. Cohen, and P.-D. Plisnier. 2002. Interpreting stable isotopes in food webs: Recognizing the role of time averaging at different trophic levels. *Limnology and Oceanography* 47(1):306–309.
- Olden JD, Mims MC, Horner-Devine MC (2013) Filtration rates of the non-native Chinese Mystery Snail (*Bellamya chinensis*) and potential impacts on microbial communities. *Limnetica*. <https://doi.org/null>

- Olden, J. D., M. C. Mims, and M. C. Horner-Devine. 2013. Filtration rates of the non-native Chinese Mystery Snail (*Bellamya chinensis*) and potential impacts on microbial communities. *Limnetica*.
- Olden, J., E. Larson, and M. Mims. 2009. Home-field advantage: Native signal crayfish (*Pacifastacus leniusculus*) out consume newly introduced crayfishes for invasive Chinese Mystery Snail (*Bellamya chinensis*). *Aquat Ecol* 43:1073–1084.
- Olsson, K., P. Stenroth, P. Nyström, and W. Granéli. 2009. Invasions and niche width: does niche width of an introduced crayfish differ from a native crayfish? *Freshwater Biology* 54(8):1731–1740.
- Pace, G.L.. 1973. The freshwater snails of Taiwan (Formosa). *Malacological Review* Supplement 1: 1- 117.
- Perron, F., Probert, T. 1973. *Viviparus malleatus*, new record in New Hampshire. *Nautilus* 87:90.
- Phillips, I. D., R. D. Vinebrooke, and M. A. Turner. 2009. Ecosystem consequences of potential range expansions of *Orconectes virilis* and *Orconectes rusticus* crayfish in Canada — a review. *Environmental Reviews* 17:235–248.
- Pianka, E. R. (n.d.). *Niche Overlap and Diffuse Competition*.
<https://www.pnas.org/doi/10.1073/pnas.71.5.2141>.
- Plinski, M., W. Ławacz, A. Stańczykowska, and E. Magnin. 1978. Etude quantitative et qualitative de la nourriture des *Viviparus malleatus* (Reeve) (Gastropoda, Prosobranchia) dans deux lacs de la région de Montréal. *Canadian Journal of Zoology* 56(2):272–279.
- Post, D. M. 2002. Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumptions. *Ecology* 83(3):703–718.

- Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montaña. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152(1):179–189.
- Pothoven SA, Madenjian CP (2013) Increased Piscivory by Lake Whitefish in Lake Huron. *North American Journal of Fisheries Management* 33:1194–1202.
<https://doi.org/10.1080/02755947.2013.839973>
- Pothoven SA, Nalepa TF (2006) Feeding Ecology of Lake Whitefish in Lake Huron. *Journal of Great Lakes Research* 32:489–501. [https://doi.org/10.3394/0380-1330\(2006\)32\[489:FEOLWI\]2.0.CO;2](https://doi.org/10.3394/0380-1330(2006)32[489:FEOLWI]2.0.CO;2)
- Pothoven, S. A., and T. F. Nalepa. 2006. Feeding Ecology of Lake Whitefish in Lake Huron. *Journal of Great Lakes Research* 32(3):489–501.
- Prestie, K., I. D. Phillips, D. P. Chivers, and T. D. Jardine. 2019. Effects of ontogeny and invasive crayfish on feeding ecology and mercury concentrations of predatory fishes. *Canadian Journal of Fisheries and Aquatic Sciences* 76(11):1929–1939.
- Q.H. GU, Q.Q. Cheng, X.J. Li and C.J. Zhou. 2015. Novel polymorphic microsatellite markers for *Bellamya* and their application in population genetics of three species. *Genetics and Molecular Research* 14(4): 15201-15212.
- Rahel, F. J., and J. D. Olden. 2008. Assessing the Effects of Climate Change on Aquatic Invasive Species. *Conservation Biology* 22(3):521–533.
- Rennie MD, Sprules WG, Johnson TB (2009) Resource switching in fish following a major food web disruption. *Oecologia* 159:789–802. <https://doi.org/10.1007/s00442-008-1271-z>
- Ricciardi A (2001) Facilitative interactions among aquatic invaders: is an “invasional meltdown” occurring in the Great Lakes? 58:13

- Ricciardi, A. 2001. Facilitative interactions among aquatic invaders: is an “invasional meltdown” occurring in the Great Lakes? 58:13.
- Ross DJ, Johnson CR, Hewitt CL, Ruiz GM (2004) Interaction and impacts of two introduced species on a soft-sediment marine assemblage in SE Tasmania. *Marine Biology* 144:747–756. <https://doi.org/10.1007/s00227-003-1223-4>
- Ruiz GM, Fofonoff P, Hines AH, Grosholz ED (1999) Non-indigenous species as stressors in estuarine and marine communities: Assessing invasion impacts and interactions. *Limnology and Oceanography* 44:950–972. https://doi.org/10.4319/lo.1999.44.3_part_2.0950
- Rundle, S. D., and C. Brönmark. 2001. Inter- and intraspecific trait compensation of defence mechanisms in freshwater snails. *Proceedings. Biological sciences / The Royal Society* 268(1475):1463–1468.
- Sandland, G. J., and D. J. Minchella. 2003. Effects of diet and *Echinostoma revolutum* infection on energy allocation patterns in juvenile *Lymnaea elodes* snails. *Oecologia* 134(4):479–486.
- Saporiti, F., S. Bearhop, D. Vales, L. Silva, L. Zenteno, M. Tavares, E. Crespo, and L. Cardona. 2015. Latitudinal changes in the structure of marine food webs in the Southwestern Atlantic Ocean. *Marine Ecology Progress Series* 538:23–34.
- Schindler, D. E., and M. D. Scheuerell. 2002. Habitat coupling in lake ecosystems. *Oikos* 98(2):177–189.
- Scott, E. (n.d.). *Globulus (Digenea), Possible Pathogens Of Dabbling Ducks In Southern Quebec*:15.

- Sheppard, K. T., G. K. Davoren, and B. J. Hann. 2015. Diet of Walleye and sauger and morphological characteristics of their prey in Lake Winnipeg. *Journal of Great Lakes Research* 41(3):907–915.
- Shi, Y., Q. Xiong, X. Wang, X. Li, C. Yu, J. Wu, J. Yi, X. Zhao, Y. Xu, and H. Cui. 2016. Characterization of a novel purified polysaccharide from the flesh of *Cipangopaludina chinensis*. *Carbohydrate Polymers* 136:875–883.
- Silberberger MJ, Koziorowska-Makuch K, Kuliński K, Kędra M (2021) Stable Isotope Mixing Models Are Biased by the Choice of Sample Preservation and Pre-treatment: Implications for Studies of Aquatic Food Webs. *Frontiers in Marine Science* 7:
- Silberberger, M. J., K. Koziorowska-Makuch, K. Kuliński, and M. Kędra. 2021. Stable Isotope Mixing Models Are Biased by the Choice of Sample Preservation and Pre-treatment: Implications for Studies of Aquatic Food Webs. *Frontiers in Marine Science* 7.
- Simberloff D, Holle BV Positive Interactions of Nonindigenous Species: Invasional Meltdown? 12
- Smith, D.G. 2000. Notes on the taxonomy of introduced *Bellamya* (Gastropoda: Viviparidae) species in northeast North America. *The Nautilus* 114: 31-37.
- Solomon CT, Olden JD, Johnson PTJ, et al (2010) Distribution and community-level effects of the Chinese Mystery Snail (*Bellamya chinensis*) in northern Wisconsin lakes. *Biol Invasions* 12:1591–1605. <https://doi.org/10.1007/s10530-009-9572-7>
- Solomon, C. T., J. D. Olden, P. T. J. Johnson, R. T. Dillon, and M. J. Vander Zanden. 2010. Distribution and community-level effects of the Chinese Mystery Snail (*Bellamya chinensis*) in northern Wisconsin lakes. *Biological Invasions* 12(6):1591–1605.
- Stańczykowska, A., E. Magnin, and A. Dumouchel. 1971. Étude 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(11):1431–1441.
- Stephen BJ, Allen CR, Chaine NM, et al (2013) Fecundity of the Chinese Mystery Snail in a Nebraska reservoir. *Journal of Freshwater Ecology* 28:439–444.
<https://doi.org/10.1080/02705060.2013.769127>
- Stephen, B. J., C. R. Allen, N. M. Chaine, K. A. Fricke, D. M. Haak, M. L. Hellman, R. A. Kill, K. T. Nemec, K. L. Pope, N. A. Smeenk, D. R. Uden, K. M. Unstad, A. E. VanderHam, and A. Wong. 2013. Fecundity of the Chinese Mystery Snail in a Nebraska reservoir. *Journal of Freshwater Ecology*.
- Stock, B. C., and B. X. Semmens. 2016. Unifying error structures in commonly used biotracer mixing models. *Ecology* 97(10):2562–2569.
- Stock, B. C., and B. X. Semmens. 2020. MixSIAR GUI User Manual. Version 3.1.
- Stock, B., B. Semmens, E. Ward, A. Parnell, A. Jackson, and D. Phillips. 2020. MixSIAR: Bayesian Mixing Models in R.
- Sura SA, Mahon HK (2011) Effects of Competition and Predation on the Feeding Rate of the Freshwater Snail, *Helisoma trivolvis*. *The American Midland Naturalist* 166:358–368
- Sura, S. A., and H. K. Mahon. 2011. Effects of Competition and Predation on the Feeding Rate of the Freshwater Snail, *Helisoma trivolvis*. *The American Midland Naturalist* 166(2):358–368.
- Therriault, T., and E. Kott. 2002. *Cipangopaludina chinensis malleata* (Gastropoda: Viviparidae) in southern Ontario: An update of the distribution and some aspects of life history. *Malacological Review* 36:111–121.
- Tilman, D. 1999. The Ecological Consequences of Changes in Biodiversity: A Search for General Principles. *Ecology* 80(5):1455–1474.

- Tornimbeni, O., R. Galvez, G. Triffault-Bouchet, N. Dassylva, and S. Roberge. 2013. Heavy metal concentrations in *Cipangopaludina chinensis* (Reeve, 1863) and relationships with sediments in Saint-Augustin Lake, Québec City (Qc, Canada). *Annales de Limnologie - International Journal of Limnology* 49(1):21–29. EDP Sciences.
- Twardochleb LA, Olden JD (2016) Non-native Chinese Mystery Snail (*Bellamya chinensis*) supports consumers in urban lake food webs. *Ecosphere* 7:e01293.
<https://doi.org/10.1002/ecs2.1293>
- Unstad, K., D. Uden, C. Allen, N. Chaine, D. Haak, R. Kill, K. Pope, B. Stephen, and A. Wong. 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.
- Unstad, K., D. Uden, C. Allen, N. Chaine, D. Haak, R. Kill, K. Pope, B. Stephen, and A. Wong. 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 Leeuwen, C. H. A., and G. van der Velde. 2012. Prerequisites for flying snails: external transport potential of aquatic snails by waterbirds. *Freshwater Science* 31(3):963–972.
- Van Mierlo V. A., Green S. J., Emmerton C. A., Nasr M., Buendia C., Wyatt F. R. and M. S. Poesch. (In Press) Occupancy of invasive Northern Crayfish (*Faxonius virilis*) in northern streams is associated primarily with water temperature. *Freshwater Biology*.
- Vander Zanden, M. J., J. M. Casselman, and J. B. Rasmussen. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. *Nature* 401(6752):464–467.

- Wang, H., D. Cai, Y. Liu, W. Zhao, F. Cai, Y. Ren, and Z. zhong Li. 2015. The effects of Roxithromycin on the Activity of APND and GST in *Cipangopaludina chinensis*. Pages 110–113. Atlantis Press.
- Wood, W. M. 1892. *Paludina japonica* Mart. for sale in the San Francisco Chinese markets. *Nautilus* 5:114–115.
- Xiong, Q., L. Zhu, F. Zhang, H. Li, J. Wu, J. Liang, J. Yuan, Y. Shi, Q. Zhang, and Y. Hu. 2019. Protective activities of polysaccharides from *Cipangopaludina chinensis* against high-fat-diet-induced atherosclerosis via regulating gut microbiota in ApoE-deficient mice. *Food & Function* 10(10):6644–6654. The Royal Society of Chemistry.
- Xu, X., J. Wu, Y. Yang, R. Zhu, and S. Yan. 2018. Operculum of a Water Snail is a Hydrodynamic Lubrication Sheet. *Journal of Bionic Engineering* 15(3):471–480.
- Yalçın Özdilek, Ş., N. Partal, and R. I. Jones. 2019. An invasive species, *Carassius gibelio*, alters the native fish community through trophic niche competition. *Aquatic Sciences* 81(2):29.
- Zhou, H., M. Yang, M. Zhang, S. Hou, S. Kong, L. Yang, and L. Deng. 2016. Preparation of Chinese Mystery Snail shells derived hydroxyapatite with different morphology using condensed phosphate sources. *Ceramics International* 42(15):16671–16676.