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Zebra Mussel (Dreissena polymorpha) Habitat Associations in Four West-Central Minnesota Lakes

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Zebra Mussel (*Dreissena polymorpha*) Habitat Associations in Four West-Central Minnesota Lakes

by
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Endorsement Date:_____

This thesis, completed by April R. Londo, has been examined and approved.

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Abstract

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In 1989, zebra mussels (*Dreissena polymorpha)* were first documented in the land of ten thousand lakes in the Lake Superior Basin at Duluth. Zebra mussels are successful invaders because the species attaches to substrates with byssal threads, can adapt to a wide range of environmental conditions, and has a free-swimming veligers that are easily transported. Although invasive mollusks pose a range of economic and ecological threats to inland waters, our understanding of zebra mussels in Minnesota lakes remains limited.

To gain additional information regarding zebra mussel ecology in lake systems, I conducted research in four west-central Minnesota lakes that were colonized prior to 2009. The objectives of this study were to 1) evaluate the relationship between zebra mussel distribution and substrate size, 2) assess the potential associations zebra mussels may have with organic biomass and individual plant species, and 3) survey the zebra mussel infested study lakes for native mussels and develop research questions about the zebra mussel and native mussel interactions.

In the summer of 2014, mussel, vegetation, and substrate surveys were completed via SCUBA at five 0.25 m^2 quadrats spread 10-m apart along six 50-m

transects in each lake. Substrate was categorized using phi (ϕ) values. Zebra mussels were enumerated and measured to determine density and size structure. Vegetative cover (%) was estimated and organic biomass was separated by type and dried to determined density (g/m^2 dry weight).

The majority (73%) of the quadrats had a phi value of 0-1, indicating small particulate substrates were available for zebra mussel attachment. Underlying geologic substrate was not a statistically significant predictor of zebra mussel density (r^2=0.32, P=0.054) in this chain of lake system, however, biological importance may be present. The study showed minimal variation in particle size among lakes within the chain-of-lake system therefore, should be considered as one unit when analyzing zebra mussel density. Significantly more zebra mussels were found attached to algaes (filamentous and *Chara* spp.) than macrophyte taxa, including *Potamogeton* spp. (P=0.001). Additionally, juvenile zebra mussels were found more on organic substrates than adults (P<0.001). Lastly, two species of native mussels were found in the study area, including fatmucket (*Lampsilis siloquoidea)* and giant floater (*Pyganodon grandis*). Future research assessing the factors that facilitate Unionid species success in their native range, compared to their introduced ranges, may help clarify the ecological mechanisms and impacts of zebra mussel naturalization.

Table of Contents

Abbreviations

Ɵ.....phi value
AIS.....aquatic invasive species
ANOVA.....analysis of variance
BCD.....buoyancy control device
C.....Celsius
Chl-*a*.....chlorophyll a
DNR.....Department of Natural Resources
DO.....dissolved oxygen
ESA.....Endangered Species Act
e.g. .....example
F.....Fahrenheit
ft.....feet
g.....grams
GPS.....Global Positioning Satellite System
ha.....hectare
i.e. .....that is
juv.....juvenile zebra mussel
km.....kilometer
LEI.....long-term effects index
m.....meter
m^2.....meter squared
m^{-2}.....per meter squared
mm.....millimeters
MN.....Minnesota
MNDNR.....Minnesota Department of Natural Resources
MNSU.....Minnesota State University, Mankato
NA.....not available
NOAA.....National Oceanic and Atmospheric Administration
NY.....New York
pH.....-log [H+]
SCUBA.....self-contained underwater breathing apparatus
SE.....standard error
spp. .....multiple unidentified species
SS.....suspended solid
Temp.....temperature
TP.....total phosphorus
U.S. .....United States
USDA.....United State Department of Agriculture
USEPA.....United States Environmental Protection Agency
µm .....micrometers

List of Tables

List of Figures

List of Appendices

1

Introduction

As a result of increased movement of people and goods, the National Oceanic and Atmospheric Administration (NOAA) estimates "since 1970, there has been on average, one invader recorded every eight months in the Great Lakes." Unfortunately, anthropogenic activity has been the main cause of intentional and unintentional exotic species proliferation (Naddafi et al. 2011; Smith and Smith, 2012). Furthermore, Cassey et al. (2005) postulated that every continent has been affected by exotic species in some capacity. Of all the non-native species to become invasive, Vrtílek and Reichard (2012) considered zebra mussels *(Dreissena polymorpha)* to be one of the most damaging.

Originally from the Ponto-Caspian region (Black and Azov Sea) in Europe, zebra mussels were unintentionally introduced into the Great Lakes at some point between 1986 (Johnson and Padilla, 1996; McMahon, 1996) and 1988 (Timar and Phaneuf, 2009). Research has suggested that zebra mussels were likely first introduced as veligers (*i.e.*, larval form) trapped in ballast water from European transiting ships (Timar and Phaneuf, 2009). Vander Zanden and Olden (2008) found that to slow the spread of aquatic invasive species (AIS), preventing secondary range expansion to smaller inland lakes and rivers becomes paramount. However, the first step in addressing AIS expansion is to understand each species invasiveness potential, population dynamics, and ecological requirements to thrive.

Zebra mussels possess several traits that make the species a highly successful invader. The primary morphological and physiological advantages include, but are not

limited to, high female fecundity males producing strong adaptable sperm, veligers capable of broad dispersal, an adult form that readily transferred due to byssal thread adhesion to substrates, and high environmental tolerances (Rahel, 2002; Timar and Phaneuf, 2009; Beyer, 2011; VrtÍlek and Reichard, 2012). Moreover, zebra mussels are able to filter a large range of particulate sizes rom the water column, have minimal predators, and maintain adaptive substrate preferences that enhances survival in nearly any environment (Lewandowski and Ozimek, 1997; Strayer et al. 1999; Zhu et. al 2006). Because of the invasive advantages of zebra mussels, the species tends to dominate colonized areas and cause a wide range of economic and ecologic impacts.

In the United States (US), invasive species cause $150 billion in damages and control efforts annually and zebra mussels alone will cost an estimated $3.1 billion over the next ten years due to their biofouling capacity (Smith and Smith, 2012). Zebra mussel impact breath are vast, and colonies of the species encrust and damage docks, boat propellers, and public services infrastructure (*e.g.*, water treatment facilities), cause human health concerns, and facilitate loss of aquatic system aesthetics (Pimental et al. 2005; McLaughlin and Aldridge, 2013; MN DNR, 2014). Furthermore, zebra mussels have also been associated with increasing aquatic plant frequency of occurrence (Zhu et al. 2006), modifications to macroinvertebrate community composition (Ricciardi et al. 1997), decreasing phytoplankton productivity (McLaughlan and Aldridge, 2013), increased frequency of toxic algal blooms (McLaughlin and Aldridge 2013), food web disruption and native mussel (Unionidae) population growth reductions

(Strayer, 1999; Aldridge et al. 2004; Zhu et al. 2006; Miehls et al. 2009, McLaughlan and Aldridge, 2013).

Zebra mussels proliferate rapidly, and established into Minnesota waters of the Great Lakes soon after the initial introduction. Although most of Minnesota remains zebra-mussel free, there are an estimated 290 of the state's 10,000+ lakes that have established populations (A. Gamble, MN DNR, personal communication), including many inland lakes (Figure 1). Although some novel research to develop alternative management applications to control zebra mussels has been conducted, no tools are currently available for effective control in natural systems.

The University of Minnesota (UM), as part of an AIS research focus, has made strides in improving our understanding of zebra mussels in Minnesota aquatic systems. For example, Dr. McCartney with the AIS center at the UM is focusing on zebra mussel genetics in hopes to sequence the species genome and gain insight about introduction patterns. Student researchers at the UM have also launched a study looking at zebra mussel attachment to three different macrophytes in Lake Minnetonka (Salverson and Zelickson, 2015). Furthermore, aggressive research and demonstration studies have been completed in Minnesota in attempts to control zebra mussels. For example, Minnesota is the first state to employ Ziquenox, a dead soil bacteria (*Pseudomonas flourescens),* in open systems as a biological control for zebra mussels (Marrone Bio Innovations, 2014).

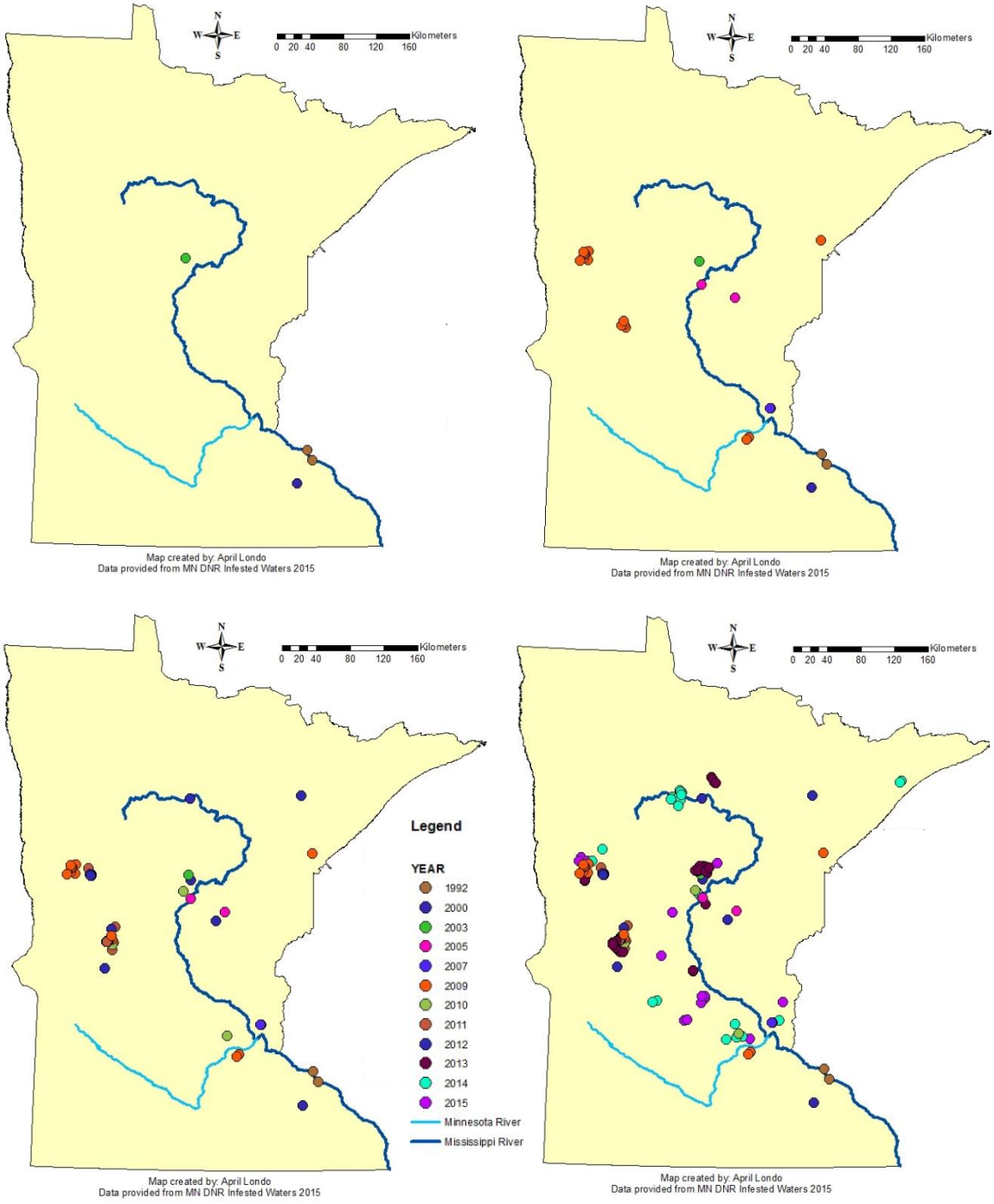

Figure 1. Progression of lakes designated as "infested" with zebra mussels in Minnesota. Colored circles denote lakes with verified depict *Dreissena polymorpha* populations and the year of first documentation. Data gathered from Department of Natural Resources Designation of Infested Waters listing updated December 16, 2013 and MN DNR data deli 2014.

Although considerable research is underway, the limited information regarding long-term zebra mussel impacts in Minnesota remains largely unknown (I. Schneider, University of Minnesota, personal communication). Minimal published research in Minnesota is available, and to my knowledge, none have assessed zebra mussel populations in west-central Minnesota. Given the importance of lakes to Minnesota's financial and ecological health, zebra mussel impacts are a concern and the development of better management practices should be a priority. However, foundational data regarding zebra mussel habitat needs (*i.e.*, geologic and vertical substrate, such as aquatic plants) and impacts on native mussels are needed.

Study Objectives

1) Evaluate the relationship between zebra mussel distribution and substrate size in lakes of Minnesota,

2) Assess the potential associations zebra mussels may have with macrophyte and plant biomass and individual plant species in lakes of Minnesota, and

3) Survey some zebra mussel infested Minnesota lakes for native mussels and develop research questions about zebra mussel and native mussel interactions.

Hypotheses

To accomplish the objectives detailed above, my research will address the following hypotheses:

H_{01}: Phi values (sediment size) will not be correlated with zebra mussel density in the study lakes,

H_{02}: Organic biomass and percent cover will have no correlation with zebra mussel density in the study lakes,

H_{03}: Zebra mussels will show no preference to differing aquatic taxa for attachment, and

H_{04}: Similar taxa of native mussels will be found in the Alexandria chain of lake system as in other Minnesota lakes.

Literature Review

Invasive Species Dynamics – An Overview

The introduction of non-native species (*a.k.a.,* exotic and alien species; Smith and Smith, 2012), that become invasive, has had wide-spread and catastrophic impacts on ecosystems worldwide (Zhu et al. 2006). The invasive species establishment rate has been estimated at 218 per one thousand years (Cassey et al. 2005). In the case of more than 1,500 exotic insect introductions in the United States since the mid-1980s, 16% have become invasive (Mooney, 2005). To become invasive, a species needs to survive the necessary steps in the *invasion process* and reproduce.

The *invasion process* of a species into a novel environment can be described by a number of successive and mandatory stages, including transport, release and introduction, and establishment (Naddafi and Rudstam, 2013). Species establishment hinges on survival to reproductive age after initial introduction. To be considered invasive, the non-native species must spread and cause harm (Keller et al. 2011). The United States Department of Agriculture (USDA), in accordance with the Endangered Species Act (ESA) of 1973, *invasive species* are "alien individuals whose introduction does or is likely to cause economic or environmental harm or harm to human health." Fortunately, most introduced species are intolerant of their new environment and perish (Smith and Smith, 2012). Although the probability for an introduced species to become invasive is low, given the sheer number of non-native species imported from their native ranges, some will succeed and often cause substantial harm to local and regional systems (Mooney et al. 2005).

Peterson et al. (2008) noted that non-native species introductions will be a primary factor in the loss of biodiversity over the next few decades. After non-native species are introduced into a new environment, a novel interaction between the potential invader and native species develops and often changes a wide range of ecological interactions (Naddafi and Rudstam, 2013). For example, when invasive species are found in abundance, negative impacts on species biodiversity and ecosystem function often emerge (Rahel et al. 2008). Strayer (1999) noted that invasive species establishment can negatively affect nutrient and contaminant cycles, performance of biological indices of water quality and other system-wide processes. Furthermore, exotic species cause large economic losses and threaten human health and welfare (Mooney et al. 2005). Not only do invasive species cause system-wide local change but alterations can also emerge on landscape and biome scales as well.

Higgins and Vander Zanden (2010) noted that species introductions are a significant menace to global diversity, and that restoring system integrity is often impossible. Island land masses, such as Hawaii, are extremely vulnerable to invasive exotic species impacts. Smith and Smith (2012) found that in the past 200 years, since non-native species were introduced Hawaii, 263 native species have disappeared, approximately 300 are listed as endangered or threatened, and an estimated 1,400 life-forms are in trouble or extinct. Hawaii is not alone, as other global regions have also experienced biological invasions that have caused significant issues.

Biological invasions are threating other global economies and ecosystems as well. For example, biological invasions are a major threat to South African biodiversity and economic livelihood. South Africa invests approximately $6.2 billion annually on terrestrial invasive plant control and management (Wilson et al. 2013). In the invaded ranges of Great Britain, Oreska and Aldridge (2011) evaluated the expansion of freshwater invaders and found the cost associated with these species to be more than $30 million annually. China's growing economy has catalyzed progressive trade practices and the country now faces exponential increase in invasive species introductions. Xu et al. (2012) estimated that from 2003-2011, China gained a total of 1,060 genera and/or species from 54 different countries, of which more than 66% were non-native terrestrial plants. In 1989, New Zealand had just as many exotic species as it did native species (Mooney and Cleland, 2001). Considering the global economic and ecological issues surrounding species introductions, the contributing factors to successful naturalization of these species needs to be better understood.

Invasiveness Mechanisms

Some biological mechanisms by which invasive species modify ecological processes include disrupting community dynamics, altering evolution trajectories, and interrupting ecosystem cycles (MacIsaac 1996; Pyšek et al. 2012). Non-native species often facilitate diseases (Rahel et al. 2008) and are vectors for parasites (Karatayev et al. 2012). It is through these novel interactions that invasive species predate on and

compete with native species that are not equipped for this disturbance (Rahel et al. 2008).

Gallardo et al. (2013) postulated that invasive species are likely to show niche expansions owing to a combination of evolutionary and ecological processes. Invasive species, in general, have a competitive advantage due to the ability to adapt to their new environments and considerable genetic variance (Elderkin et al. 2004) that facilitates a broad range of exploitative phenotypes. Furthermore, non-native individuals have minimal predators in their new environment, allowing for sequestration of energy; therefore, devoting more of non-native species' resources for growth, making them more successful (Mooney et al. 2005).

Hierro et al. (2005) found that factors that allow invasive species to become abundant, include, but are not limited to:

- high abundance in original range,
- polyphagous feeding behavior,
- short generation time,
- genetic variability among the introduced individuals,
- fertilized females able to colonize alone,
- larger than most related species,
- associated with humans,
- able to function in a wide range of physical conditions,
- attributes of high dispersal rate,
- single-parent reproduction,
- phenotypic plasticity,
- large native range,
- eurytrophy, and
- human commensalism.

Invasive traits allow some non-native species to capitalize on novel environments that may include empty, or partially empty niches. The more traits an individual AIS possesses from the list above, the better suited to be a successful invasive species. It is important to recognize, however, that some invasive species exhibit many of these traits while others only exhibit a few. An invasive species is one that is very successful and has a wide variety of mechanisms facilitating their ability to survive in invaded ranges – a process often expedited by anthropogenic influence.

The Anthropogenic Role in Species Invasion

In native ranges, invasive species are not generally "invasive" and are mediated by ecological limitations (Hierro et al. 2005). When species are moved outside native ranges, their abundance can be influenced by a different set of factors (Hierro et al. 2005) that can result in a niche expansion (Gallardo et al. 2013). Humans have facilitated non-native introduction into novel ranges due to the increase in trade and travel (Mooney et al. 2005). Humans have played a significant role in species invasions through both intentional and unintentional spread of species beyond their natural range (Naddafi et al. 2011; Smith and Smith, 2012). Subsequently, humans are both indirectly and directly responsible for the majority of non-native species introductions of (Timar and Phaneuf, 2009). In fact, Cassey et al. (2005) noted that one of the most prevalent and persistent factors in anthropogenic global change is the introduction of exotic species. Furthermore, the disruption of natural landscapes, increase in global

exportation of goods, and climate change, have created a scenario in which invasive species are more successful than ever before (Mooney et al. 2005).

Many problematic invasive species were introduced to new ranges intentionally. Smith et al. (1999) found that many invasive pests have been introduced as the result of legal importation to satisfy the demands of the pet trade, aquacultural production, decorative landscaping, and agricultural industry (Smith et al. 1999). Numerous species, for example, have been introduced as pets. In the past 30 years, more species are being traded among countries than ever before, ultimately, expediting the rate of invasions (Wilson et al. 2013). Consequently, the purposeful transport of species is contributing to great economic damage (Mooney et al. 2005).

However, many invasive species have also been introduced unintentionally, by several vectors. In aquatic environments, the majority of species introductions in both Europe and North America have been via ballast water discharge (Beyer et al. 2011). Beyer et al. (2011) also found that organisms attached to boat/ship hulls facilitated the successful invasion of over 130 species in the Ponto-Caspian region in Europe and more than 180 species in the Great Lakes of North America. Additionally, Bax et al. (2003) found that at every given moment, approximately 10,000 different species are being transported between bio-geographic borders via ballast water tanks alone. The secondary spread by water currents, flooding, attachment to animals or transport in internal organs of fish occur, further exasperating the problem (Havel and Shurin, 2004;

Havel and Medley, 2006). However, anthropogenic mechanisms are the leading cause of many AIS transfers (Beyer, 2011).

Not only do human activities transport species, but human-modified habitats and increasing global temperatures humans are also directly helping invasive species establish. For example, Hierro et al. (2005) found that increases in water levels and nitrogen availability, both of which are anthropogenic-driven changes, enabled zebra mussel invasions. With the construction of roads, dams, and channels, the distributional range expansion of numerous species has also been facilitated (Hierro et al. 2005). Invasive species come well equipped to tolerate environmental extremes, and therefore, as human activity modifies habitat conditions, non-native species can often proliferate (McMahon, 2002). Biological invaders are a universal threat to ecosystems and zebra mussels rank among the world's top 100 most bothersome and damaging invasive species (Aldridge et al. 2004; Miehls et al. 2009; Higgins and Vander Zanden 2010; VrtÍlek and Reichard 2012).

Zebra Mussel Species Profile

Zebra mussels belong to the Kingdom Animalia and are part of the Phylum Mollusca because they consist of a hard outer shell and soft inner portion (soft tissue; Invasive Species Compendium, 2015). The species belongs to the Class Bivalvia because the shell consists of two parts and is in the Order Veneroida because of similar characteristics as marine bivalves. The Family of zebra mussels is Dressenidae because they are "platform mussels" of brackish water that possess byssal threads for

attachment. The zebra mussel genus is *Dreissena* and species is *polymorpha* (Invasive Species Compendium, 2015).

Zebra mussels originated in eastern and central Europe (Timar and Phaneuf, 2009), where the species has a broad geographic range across the Caspian, Black, and Azov seas (*a.k.a.,* the Ponto-Caspian region; Strayer and Smith, 1993). Although zebra mussels have been studied for almost 200 years, few assessments have addressed invasiveness and naturalization mechanisms. Much of the zebra mussel research has been done in Eastern Europe and areas of the former Soviet Union, and due to limited translations of these works into English, many western researchers cannot utilize the information (USEPA, 1991). Of those who have researched native and introduced zebra mussels, comparative populations dynamic studies have been addressed showing thermal, saline and density difference among zebra mussel cohorts and among regions.

Aldridge et al. (2004) postulated that due to differing temperature regimes and salinity across ecoregions, zebra mussels might exhibit substantial changes in phenotype. Additionally, a Europe-North America zebra mussel comparative study, found that North American populations had higher thermal tolerance than their European ancestors (McMahon, 2002). Gallardo et al. (2013) found that North American zebra mussels had a broader bioclimatic niche for extreme temperatures (*e.g.*, up to 16 °C higher annual temperature ranges) than those in the Ponto-Caspian regions and Europe. Gelembiuk et al. (2006) suggested that the North American zebra mussel higher tolerances were due to *Dreissenid* mussel evolution in dynamic and unique

environments temporally, ultimately, predisposing them to becoming invasive. Thus, an inference can be made that based on local conditions, North American zebra mussels can adapt widely to thermal varieties (Beyer, 2011).

In addition to the evolutionary advantage of thermal adaptation, Gallardo et al. (2013) found that zebra mussel success has also been catalyzed by the species saline tolerance, and the similarities between native and invaded ranges. Furthermore, zebra mussels now occupy a broad range of ecological boundaries (*e.g.*, North America, Pont-Caspian region, and much of Europe), allowing for adaptations to become established in each region, separate from one another, and facilitating the acclimation of this invasive species (Gallardo et al. 2013). With the zebra mussels' capacity to acclimatize in introduced ranges, the species can often reach higher densities. For example, Naddfi et al. (2011) found the claim made by Hierro et al. (2005) that translocated invasive plant populations will occur at greater densities and have greater fitness than in native ranges, held true for zebra mussels as well.

Zebra Mussel Distribution and Expansion

Dispersal

The geographic range of zebra mussels has greatly expanded from its native range in the Ponto-Caspian region, now including numerous lake and river systems in both Europe and North America (Strayer and Smith 1993; Timar and Phaneuf 2009). In the 19th century, canal construction enabled zebra mussels to freely spread from land-locked areas to previously unavailable habitats across Europe and the Mediterranean to

the Arctic Circle (Strayer 1999; Higgins and Vander Zander, 2010) and eventually to North America.

Zebra mussels were first detected in North America in 1988 in Lake St. Clair, near Detroit, however, based on morphology and size structure Johnson and Padilla (1996) and McMahon (1996) believe they were introduced in 1986. Regardless of introduction date, the exact origin of the larvae is not known (Strayer, 1999; Berkman et al. 2000; Timar and Phaneuf, 2009). Within two years after the spread in Europe, zebra mussels were detected in all five of the Great Lakes and soon after entered and spread throughout the Mississippi watershed (Timar and Phaneuf, 2009). The introduction of zebra mussels into other parts of Europe and North America was then further facilitated by human activity, resulting in broad dispersal and frequent proliferation (Naddafi et al. 2011; Smith and Smith, 2012).

Physiology and Reproduction

Most adult zebra mussels are about the size of a fingernail, but can grow to a maximum length, when measured dorsal margin to umbo carapace, of 50 mm and have a life expectancy of four to five years (Timar and Phaneuf, 2009). Zebra mussels get their species name *polymorpha* due to the many morphotypes that all resemble zebra stripes (Invasive Species Compendium, 2015). The diploid number for zebra mussels is 32, and have been found under laboratory studies to hybridize with *D. bugensis* (a relative of the zebra mussel) (Invasive Species Compendium, 2015).

Similar to a trait shown by marine bivalves (Kobak, 2000), fertilization can occur as dioecious adults shed gametes into the water column at temperatures >10° C (Strayer, 1999). Dependent on water temperature, veligers are created after fertilization in May and June and usually grow rapidly through October (Muskó and Bankó, 2005). Depending on food availability and water temperature (Strayer, 1999), veliger development can take 1-9 weeks, grow to 80-220 μm in length and feed on small phytoplankton (Martel et al. 1995). The biology of zebra mussels is comparable to most AIS, but do have many unique characteristics that enhances proliferation.

Zebra Mussel Invasiveness and Plasticity

Given zebra mussels rapid expansion into parts of Europe and North America, the species capitalizes on many features described above that enable invasiveness. VrtÍlek and Reichard (2012) suggested that zebra mussel invasions are facilitated by the absence of an ecological equivalent in freshwater systems and the species extremely high fecundity, but there are many other factors that also contribute to zebra mussels' success. Researchers have collectively identified several zebra mussel traits that allow the species to be highly invasive, including strong sperm viability, a planktonic larval stage (veliger) capable of effective dispersal, adult form that can also be easily transferred, adaptive substrate preference and high tolerance of environmental factors (*e.g.*, Rahel, 2002; Timar and Phaneuf, 2009; Beyer, 2010; VrtÍlek and Reichard, 2012).

Broadcast spawners generally have a disadvantage due to the dilution of their sperm in freshwater, possibly limiting fertilization (Levitan 1993) but this is not the case

of zebra mussels. Zebra mussels are "r" strategists with a short maturation time (1-2 years) and a high fecundity (> 1 million eggs produced per female per spawning event) (Strayer, 1999; Invasive Species Compendium, 2015). Furthermore, zebra mussel eggs releases chemoattractant signals for the traveling sperm (Miller et al. 1994; Ciereszko et al. 2001) and both sexes exhibit synchronistic spawning (Hardege et al. 1997). Fertilization success is directly related to an animals' fitness (Levitan 1993). Quinn and Ackerman (2012) found that zebra mussels had higher sperm potency compared to other broadcast-spawning animals throughout the world [*e.g.,* blood cockle (*Tegillarca granosa*) of the Indo-Pacific region and crown-of-thorns starfish (*Acanthaster planci*) from Australia] that facilitated successful fertilization under low initial population densities. In comparison to other freshwater animals, such as fish, zebra mussels' spermatozoa have remarkable viability and their motility may be the longest among freshwater animals (Ciereszko et al. 2001). Quinn and Ackerman (2012) also concluded that zebra mussel sperm potency catalyzed successful fertilization in a range of flow patterns and water concentrations (Quinn and Ackerman, 2012).

The byproduct of fertilization is a microscopic larvae called a veliger. This life stage is very important in the overall success of this species as an invasive. Veligers are planktonic, making them easily transported (Johnson and Padilla 1996). It has been postulated that the first zebra mussels introduced to North America were transported as veligers in the ballast water of a cargo ship that originated its voyage in Europe (Timar and Phaneuf 2009). Furthermore, evidence indicates that a primary overland vector of

zebra mussel dispersal is the unintentional transport of veligers in the bilge, cooling water, live wells, and bait buckets, of transient recreational vessels (Timar and Phaneuf, 2009). Biologically, these plankotrophic larvae exploit areas of the water column by consuming food resources in the photic zones (MacIsaac et al. 1992). Veligers require weeks for development in the plankton allowing sufficient time for widespread dissemination and movement via currents and wind (Johnson and Carlton 1996).

Juvenile zebra mussels settle out of the water column in search of reliable substrate (Kobak, 2000), but scientists are now finding that zebra mussels can utilize a broader range of substrates than previously believed, increasing the likelihood of successful colonization (Bonner and Rockhill, 1994; Ricciardi et al. 1997; Farsad and Sone, 2012). Zebra mussels often colonize areas with hard substrate in North America and Europe (Mellina and Rasmussen, 1994), however, Berkman et al. (1998) discovered a colonization strategy in Lake Erie where zebra mussels inhabited several hundred square kilometers of sand and mud substrate. Larval and juvenile *Dreissenids* can attach directly to small sediment particles (Berkman et al. 1998), a life history strategy often utilized by marine bivalves (Stanley, 1972). The Berkman et al. (1998) discovery was a diversion from the accepted zebra mussel colonization strategies because it was demonstrated that the species has the ability to colonize soft sediment by the use of many sand particles as a “seed” for byssal attachment (Berkman et al. 1998; Berkman et al. 2000).

Veligers are not the only zebra mussel life form to be transported. Both forms (veliger and adult) of the zebra mussel can be easily transported and once matured adults, can adhere via byssal threads to various substrates, including unionid shells, stones, macrophytes, and human-made structures like breakwaters, pipes and boats (Johnson and Carlton, 1996; Strayer 1999; Timar and Phaneuf, 2009). Human activity, such as recreational boating and global commerce, has directly enhanced physical veliger and adult dispersal (Timar and Phaneuf, 2009). Additionally, anthropogenic-driven increases in global temperature have indirectly improved zebra mussel invasiveness because of warmer waters and higher sea levels that have made more habitat area accessible to and suitable for zebra mussel naturalization. In addition to behavioral and physiological traits that improve zebra mussel colonization success, the species also has important physiological advantages that enhance their reproductive capacity.

Like most ectothermic species, zebra mussel distribution is affected greatly by water temperature (Rahel, 2008). Additionally, it has been shown by Gallardo et al. (2013) that zebra mussels have tolerated a broad range of different environmental factors over space and time to allow for range expansion. Zebra mussels are able to flourish in polluted waters, giving them a competitive edge over native species (Hamilton, 2010). Zebra mussels exhibit behavioral plasticity that enables the species to survive and proliferate in many disturbed areas (*e.g.*, tolerances for varying substrates), an advantage not normally seen in other native freshwater fauna. (Hamilton, 2010). The

collective result of a macroscopic life stage, strong sperm attributes, capacity to utilize diverse substrates, and water quality tolerances and have been recognized as key factors in zebra mussel invasiveness (Quinn and Ackerman, 2012).

Zebra Mussel Ecology

As outlined above, zebra mussels are an effective invader. Zebra mussels also have a unique feeding behavior, high tolerances to environmental parameters (*i.e.*, pollutants; Padilla et al. 1996; Timar and Phaneuf, 2009) and minimal predators (Naddafi and Rudstam, 2013). Zebra mussels can form dense beds (Kovalak et al. 1993) and are substrate generalists (Ricciardi 1997; Berkman et al. 1998), including attachment to vegetation (Lewandowski and Ozimek, 1997).

Feeding

Adult zebra mussels remain stationary while filter feeding, allowing them to process up to 1 L of water per individual per day (McLaughlan and Aldridge, 2013; Timar and Paneuf, 2009). For example, this filtering ability meant that zebra mussels in Lake St. Claire were able to filter the entire lake several times a day (USEPA, 1991). Zebra mussels are able to remove particles ranging in size from <0.2 mm in diameter to filamentous algae as large as 1.2 mm in diameter (Kraemer et al. 2013). Algae and other particulates are ingested into the zebra mussel via an inhalant siphon (Figure 2), but not all matter that enters is ingested (McLaughlan and Aldridge, 2013). Unpalatable materials are rejected by the zebra mussel as mucus-bound undigested material expelled by the inhalant siphon called pseudofeces (Stanczykowska et al. 1975).

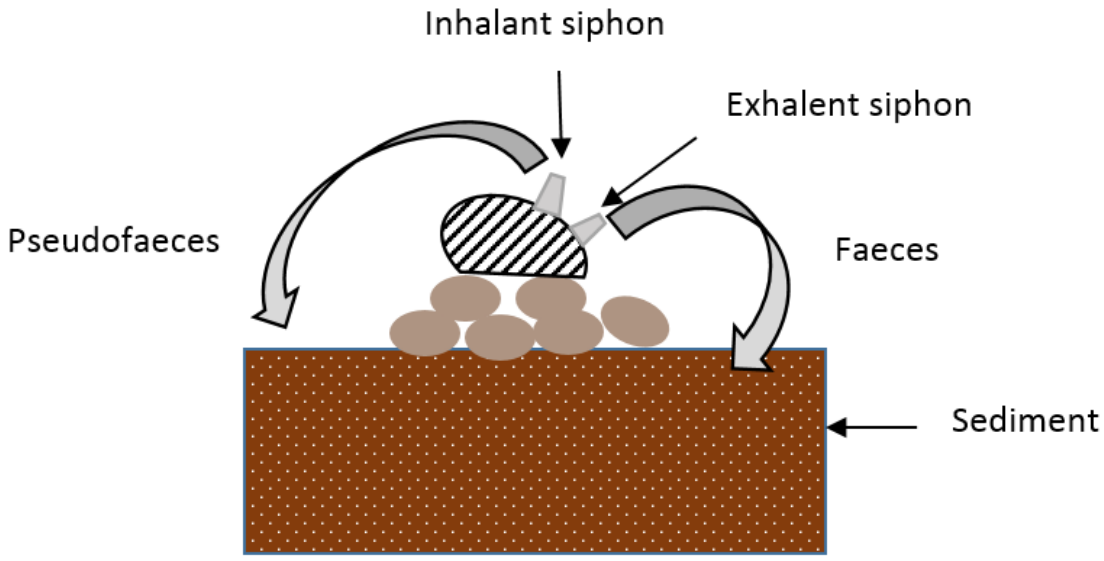

Figure 2. The mechanism by which zebra mussels ingest particulate. Particulate is taken into the inhalant siphon. Undigested material is packaged in mucus by the zebra mussel and is excreted as pseudofeces by the inhalant siphon. Undigested material that has passed through the gut is then excreted by the exhalant siphon as negatively buoyant feces. Both forms of feces are then returned to the sediment. (Adapted from Stanczykowska et al. 1975.)

Tolerances

Zebra mussels have the ability to colonize water bodies with a wide range of chemical and physical properties, and can survive 3 to 7 days of aerial exposure (Padilla and Johnson 1996; Timar and Phaneuf, 2009). Survival parameters that have been extensively studied include pH, conductivity, total hardness, alkalinity, temperature, salinity levels, sulfate and calcium. Veliger and adult zebra mussels are able to survive a broad range of parameters (Table 1). Zebra mussels have few limitations that impede growth and reproduction. However, low water temperature and some environmental pollutants (*e.g.*, cadmium) limit reproduction and growth (deKock and Bowmer 1993). Naddafi et al. (2011) found higher densities of zebra mussels in larger lakes than in stream and rivers. In aquatic ecosystems, zebra mussels inhabit depths of 2 to 7 meters (Timar and Phaneuf, 2009). Zebra mussel abundance has been reviewed as a function of the strata on which they settle, with orders of magnitude more individuals found on hard substrate compared to sand or mud (Higgins and Vander Zanden 2010).

Predators

Molloy et al. (1997) evaluated natural predators of zebra mussels and found many different species present in Europe that are not in the zebra mussels invaded ranges. Fish with molariform pharyngeal teeth can grind and crush molluscs (Molloy et al. 1997). Although some molluscivores in North America exist (*e.g.*, Freshwater Drum *Aplodinotus grunniens*), predators have not demonstrated a capacity to impact zebra mussel growth and reproduction. Roach (*Rutilus rutilus), are* the most effective predator

Table 1. Environmental parameters and threshold limits above which zebra mussels can survive. Table accumulated from multiple sources. Symbols denote authors who did primary research.

Parameter	Adult	Veliger
pH*¥	>6.9	>8.5
Conductivity (µS)*	>22	
Total hardness (ms CaCO3/L)*¥	>23	>64
Alkalinity (mg $CaCO_3$/L)*¥	>18	>40
Temperature (°C)¶	<31	
Salinity level[Θ£]	10%	4.5%
Sulfate[Θ]	high	
Calcium (mg/L)[Θϵ]	3-8 to high	>13

Note: for Temperature there was 100% mortality at 36 °C
* Claudi and Mackie, 1993
¶ Beyer, 2010
Θ Wright et al. 1996
ϵ Mellina and Rasmussen 1994
¥ Hincks and Mackie 1997
£ Kilgour et al. 1994

of veligers, juvenile and adult zebra mussels, but the species is not present in North America. Roach are very effective at scraping surfaces dense with zebra mussels. Other predator species include Redear Sunfish (*Lepomis microlophus*), Freshwater Drum (*Aplodinotus grunniens*), Sturgeon species (family Acipenseridae), some suckers (family Catostomidae) and cyprinids, crayfish, blue crabs (*Callinectes sapidus*), turtles, coots (*Fulica* spp.), and diving ducks (Molloy et al. 1997). Additionally, Naddafi and Rudstam (2013) found that Pumpkinseed Sunfish (*Lepomis gibbosus*) and rusty crayfish (*Orconectes rusticus*) are effective consumers of zebra mussels. Although these successful predators may devour large numbers of zebra mussels on a local scale, they only exert temporary control in zebra mussel populations in invaded ranges (Strayer, 1999). Round Goby (*Neogobius melanostomus)*, another invasive species to North America, was found by Naddafi and Rudstam (2013) to consume the most zebra mussels.

Habitat Selectivity, Substrate and Density

Habitat Selectivity

Zebra mussels have the ability to adhere to a multitude of substrates but are seen in higher densities on some over others; with the selection of this substrate being important for survival (Oldham, 1930; Kobak 2000; Porter and Marsden, 2008). Much like marine bivalves, juvenile zebra mussels use mucous threads (Figure 3) to float in the water column until they are heavy enough and ready to settle (Mackie and Schloesser, 1996; Kobak, 2000). Over a period of weeks (Oldham, 1930), settled out zebra mussel juveniles crawl over substrate until reaching a suitable site for attachment - a decision

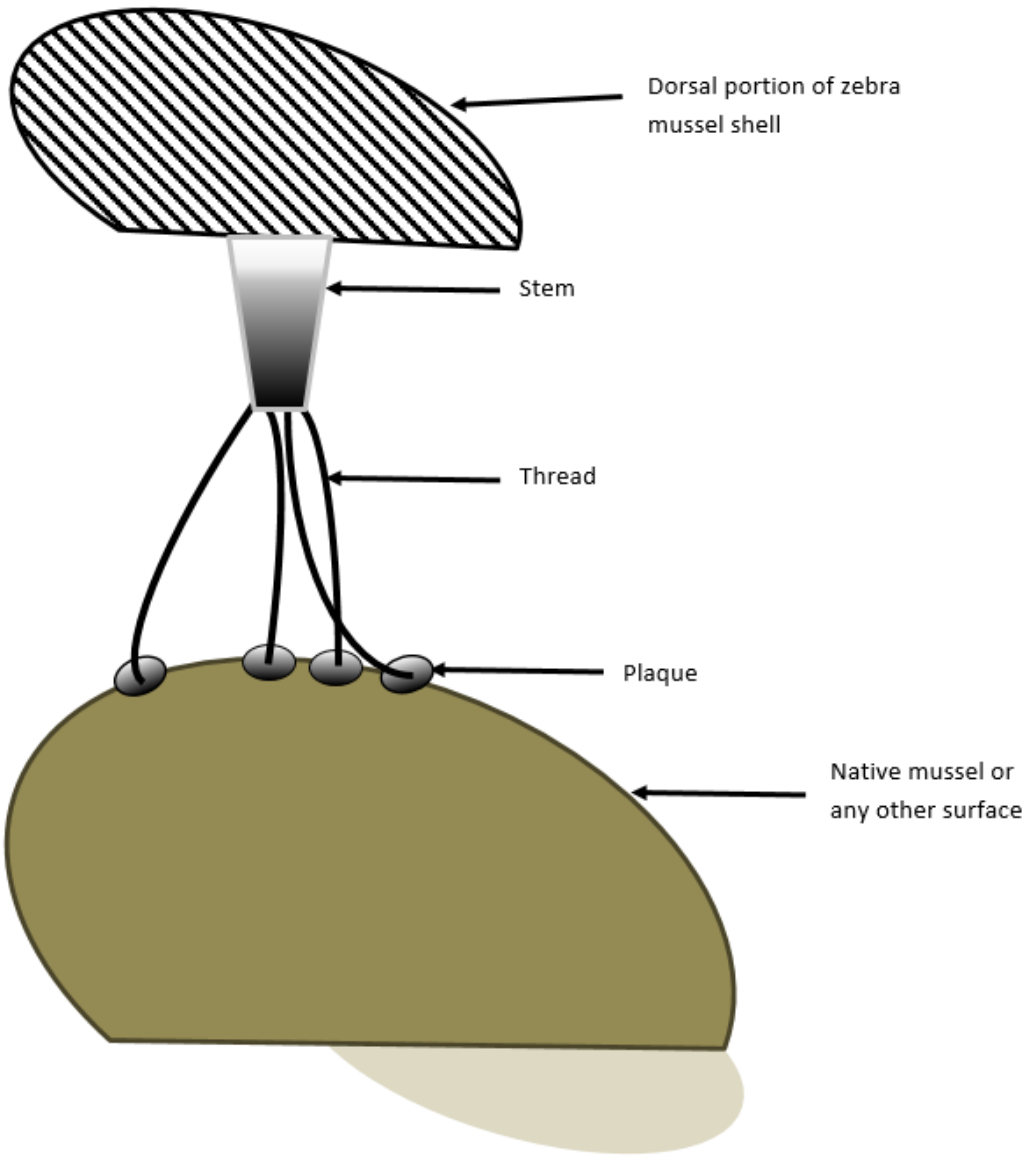

Figure 3. Schematic drawing of byssal apparatus, displaying the stem that is attached to a retractor mussel, the thread that allows the zebra mussel to float and the plaque that attaches directly to the substrate. Not drawn to scale. (Adapted from Farsad and Sone 2012.)

that may be more important than previously thought (Kobak, 2000). The crawling action of a zebra mussel is known to be triggered by one abiotic resource, light (Kobak, 2000); but other factors are may also influence zebra mussel movement. Kobak (2000) suggested that zebra mussels may exhibit negative phototaxic behavior. He found both adults and juvenile zebra mussels sought out dark zones of petri dishes and showed preference for lower parts of test-tubes (Kobak, 2000). The selection of darker regions may be associated with stones, rocks and crevices seen by zebra mussels to be refuges from predators, dislodging and desiccation (Kobak, 2000).

Detachment

If a site selected by a zebra mussel is not ideal, individuals can detach byssal mass (Oldham, 1930) and crawl 7 cm/night for juveniles and 36 cm/h for adults (Toomey et al. 2002). Porter and Marsden (2008) found in less than a day an adult zebra mussel can detach and relocate to a new site. Although zebra mussels are able to relocate, the decision made includes a trade-off in using the energy for re-attachment of byssal threads and the possibility of finding new substrate on the other, a relocation made more by smaller zebra mussels than larger (Kobak, 2000).

Deterrents

Not all substrates are used for attachment by zebra mussels; a few are shown to be deterrents for this invasive species. Porter and Marsden (2008) found mesh is not a suitable substrate, and in the presence of only mesh zebra mussels adhere to each other rather than the synthetic surface. Ultraviolet light has been used in intake pipes due to its toxic properties to veligers (D. Jensen, University of Minnesota, personal

communication). The removal of zebra mussels from intake pipes can be facilitated using antifouling paints and silicone coating that decreases byssal attachment strength and the initial settlement can be deterred using toxic coating such as copper, tin and zinc (Porter and Marsden, 2008; Ranschaert and Maxson, 1995). Nevertheless, some of these paints are banned in some inland waters due to their toxic impacts on humans and such uses may not be appropriate for all circumstance (Porter and Marsden, 2008).

Optional abiotic substrate

Zebra mussels have the ability to colonize soft substrates (S. McComas, personal communication), although this is not optimal (Ricciardi 1997; Berkman et al. 1998). To colonize soft sediment, the zebra mussel usually needs a "seed" surface such as a dead zebra mussel shell or pebble to use as a foci for aggregation of byssal thread attachment (Ricciardi et al. 1997). Even one pebble can allow a mussel to adhere, creating a surface other mussels can then attach to, forming 2-3 layers thick of zebra mussels (McComas et al. 2014). Conversely, Berkman et al. (1998) found Lake Erie zebra mussel assemblages lacked any hard substrate or "seeds" for attachment, but still were able to survive. These observations suggest zebra mussel have the ability to bind sediments using their byssal threads and directly colonize sand substrates (Berkman et al. 1998).

Zebra mussels can also attach to each other, forming dense colonies with over 10,000 mussels m^{-2} and up to 0.3 m thick (Bonner and Rockhill, 1994). Additionally, they have the ability to attach to other surfaces such as metal and synthetic materials. Farsad and Sone (2012) found the strongest zebra mussel attachments were to rough natural substrates, and the weakest attachments were to smooth polymeric substrates.

Therefore, zebra mussel attachment strength varies with substrate type (natural> metallic >polymeric), material composition and substrate roughness (Farsad and Sone 2012).

Optimum substrate

Although zebra mussels can adhere to many different substrates, it is assumed the species still has preferences. When metamorphosed, juveniles pick a substrate where assumed optimal conditions include hard substrate comprised of rock and woody structures for byssal attachment and good water chemistry (McComas et al. 2014). Natural structures such as stone was favored over treatment bricks (Ricciardi et al. 1997) in the St. Lawrence River from 1994-1995, finding 50% lower density on the brick substrate. Zebra mussel densities have been noted to vary significantly with the size of their underlying hard substrates (Mellina and Rasmussen, 1994; Berkman et al. 2000), showing a decrease in density as the sediment size decreased in grain size to vary with the size (Berkman et al. 200). Substrate size has been analyzed in past studies of other infested areas, indicating a distinct correlation between substrate size and depth with zebra mussel density (Mellina and Rasmussen 1994; Berkman et al. 2000). Naddafi et al. (2010) noted that the zebra mussel densities were greatest in areas 2-m deep with large particle size substrates. Furthermore, Mellina and Rasmussen (1994) found substrate size alone accounted for 38-91% of variability in zebra mussel density in three aquatic systems. The correlation between zebra mussel density and substrate size has been extensively documented in single lakes and in river systems but, to my knowledge,

has not been addressed in a chain of lake system where hydrology and geomorphology are similar.

Vegetation as a reliable habitat

In addition to geological substrate influence on zebra mussel density, densities may also be influenced by the relationship of plants and light intensity/shading, but until now, there has been only limited research on this relationship. As a result of zebra mussel water column filtering capacity, water clarity increases and aquatic plants are able to thrive and grow to deeper depth (Zhu et al. 2006). With a greater biomass of plants growing in systems with zebra mussels, there are ultimately more preferred shaded areas for the negatively phototaxic zebra mussels to inhabit (Toomey et al. 2002). This avoidance behavior with light levels may be advantageous to zebra mussels and their development (Toomey et al. 2002). Similarly, Kobak (2000) found an inverse correlation between light penetration and the density of zebra mussels. Not only do zebra mussels attach to aquatic plants (Ozimek, 2007; Lewandowski and Ozimek, 2007), the shade created may create preferred spaces for zebra mussel development, however, this theory has not been evaluated.

Because zebra mussels can adhere to a wide range of substrate types, including aquatic macrophytes, macrophytes should be considered when predicting zebra mussel densities. Although submerged vegetation is not a homogenous or sturdy substrate, Lewandowski and Ozimek (1997) found that macrophytes are suitable for settling

juvenile zebra mussel attachment. Macrophytes, according to Ozimek (1997), also provide a good source of food, detritus and algae.

Stanszykowska and Lewandowski (1993) argued that zebra mussels can occur in higher densities on submerged macrophytes than on other substrata, such as sand and silt. Additionally, Muskó and Bakó (2005) found evidence that the highest density of zebra mussels in a Hungarian Lake was associated with the highest density of macrophytes. Basic understanding, however, of zebra mussel association with macrophytes as habitat is lacking.

Although all aquatic macrophytes can be utilized by zebra mussels as suitable substrate, Ozimek (1997) found that the most suitable macrophyte species were seasonal (perennial) and had long-term stability, branching structure of shoots forming large surfaces for settlement and dense with refuges from predators. Additionally, Lewandowski and Ozimek (1997) found the abundance of zebra mussels is affected by how many shoots a macrophyte has, as well as substrate – with the best case being areas with dense growth of macrophyte. The region of the plant in which a juvenile zebra mussel settles is also important. Lewandowski and Ozimek (1997) found that zebra mussels settle more densely at the base of leaves of coontail (*Ceratophyllum demersum)*, than in uncovered spaces, suggesting a potential preference for areas on a plant as well as the species of plant as a substrate (Ozimek 1997).

The type of plant that a zebra mussel settles on and attaches to is also important. *Chara* spp., a type of macroalgae, has been shown to be a primary organic

substrate of choice for zebra mussel attachment (Ozimek, 1997, Lewandowski and Ozimek, 1997). *Chara* spp. forms dense mats, providing ideal areas for zebra mussel to attach in the interior portions to avoid predation (Ozimek, 1997). Furthermore, *Chara* spp. are usually viable for more than two years, allowing the zebra mussel to conserve energy for growth and minimize energy use for relocation (Ozimek, 1997). Additional species identified as optimal for zebra mussel attachment are *Ceratophyllum demersum*, *Nitellopsis* spp., and *Stratiotes aloides* (Table 2; Lewandowski and Ozimek, 1997).

Population Dynamics

Zebra mussels can form dense beds consisting of thousands of individuals per square meter (Kobak, 2000). Zebra mussel density has been shown to have an inverse relationship to the general health and condition of zebra mussels (Hunter and Bailey 1992) and the damage caused by this invasive species is strongly correlated to population size (Naddafi et al. 2010). Moreover, length-frequency distribution (*i.e.*, size-structure) is a valuable tool in determining zebra mussel growth and survival of individual cohorts, ultimately offering information regarding the effects of this exotic species on ecosystems (Naddafi et al. 2010).

Understanding density, population dynamics, habitat preferences and distribution are important factors in efforts to manage zebra mussels. Although there has been significant monitoring for zebra mussels in areas such as Lake Pepin and Mille Lacs Lake in Minnesota, there are minimal density surveys being done in the state (G. Montz, MN DNR, personal communication). Furthermore, determining species

Table 2. Table modified from Lewandowski and Ozimek (1997) showing the maximum numbers if Dreissena polymorpha on different macrophyte taxa in the Lake Majcz Wielki in 1994. More plant species were documented but not all are included in the table due to negligible attachment.

Macrophyte	Number of zebra mussels (indiv. m^{-2})
Chara spp.	2892
Ceratophyllum demersum	1750
Nitellopsis obtusa	975
Myriophyllum spicatum	196
Elodea canadensis	182

distribution, abundance and structure is a major goal of ecological research (Naddifi et al. 2010). Mellina and Rasmussen (1994) highlighted that a critical factor in the design and implementation of a control program is understanding abundance and distribution of zebra mussels. Moreover, more research needs to be done to explore factors that regulate zebra mussel density and populations on a local scale (Naddafi et al. 2010).

Zebra Mussel Impacts

Economic

The greatest economic loss caused by zebra mussels is the direct result of their ability to colonize any hard substrate (Connelly et al. 2007). As zebra mussels colonize water intake pipes used for electric power generation, water treatment, irrigation, and a range of industrial applications, the invaders can reach densities that block water flow, causing substantial economic losses due to down time and structural damages (Timar and Phaneuf, 2009; O'Neill 1997). The U.S. Geological Survey reported economic loss caused by zebra mussels from 2000-2010 was as much as $5 billion in the Great Lakes region alone (Timar and Phaneuf, 2009).

To alleviate structural damage, industrial facilities have tried to control zebra mussel blockages, which in 1995 was already exceeding $17.7 million in expenses at 339 facilities annually (O'Neill, 1997). Consequently, control for the bio-fouling of water intake pipes and power facilities is a serious problem; costing North America an estimated ~$267 million (from 1989 to 2004), with an ongoing cost of ~$11-$16 million dollars per year (Connelly et al. 2007, Higgins and Vander Zanden, 2010).

Although there is no finite information regarding economic loss in Minnesota, there have been reports conducted on local levels. Douglas County Commissioner's Citizens' Committee on Zebra Mussels reported that economic and ecological losses are still being revealed, but that evidence suggests fish populations have declined. Alexandria is a community in west-central Minnesota that relies heavily on the tourism industry based on year-round fishing and seasonal recreation (Douglas County Commissioner's Citizens' Committee on Zebra Mussels, 2011). Fishing tournaments in the area attract thousands of boaters each year and is an important component of the local and regional economy (Douglas County Commissioners' Citizens' Committee on Zebra Mussels, 2011). Furthermore, zebra mussels decrease aesthetics of aquatic systems, ultimately deterring people from not only Douglas County, but other places in Minnesota as well. No state-wide estimates on the economic losses caused by zebra mussel infestations has been completed in Minnesota (G. Montz, MN DNR, personal communication). Measuring the impacts of zebra mussels, however, goes well beyond dollars.

Ecological

Not only do zebra mussels cause billions of dollars in damages in the United States (Timar and Phaneuf, 2009), this species can also facilitates extraordinary biotic and abiotic changes to aquatic habitats (Scheffer et al. 1993; Zhu et al. 2006; McLaughlan and Aldridge, 2013). The breadth and extent of zebra mussel impacts does include both positive and negative changes (Scheffer et al. 1993). Zebra mussels

influence macrophytes, invertebrates, plankton, and native mussels, through indirect changes catalyzed by abiotic interactions (Zhu et al. 2006) and direct competition for resources (Aldridge et al. 2004). Zebra mussels can be described as ecosystem engineers for their extreme ability to alter the structure and function of systems, ultimately posing considerable threats to food webs (Zhu et al. 2006; Miehls et al. 2009). System-level changes caused by zebra mussels has expedited the extinction of many aquatic species (Aldridge et al. 2004).

The most prominently documented consequence of zebra mussel colonization is the increase in water clarity through the sequestration of phytoplankton (algae) and particulate from the water column (Zhu et al. 2006). Higgins and Vander Zanden (2010) reported a 38.5% increase in average lake secchi depths and an increase of 50.5% in the littoral zone secchi depth reading after the introduction of zebra mussels (Table 3). Scheffer et al. (1993) found that, in a reservoir, there was a feedback loop created by zebra mussels to aid in the shift from a turbid state to a clear state (Figure 4).

Higgins and Vander Zanden (2010) also found that turbidity, TP, SS and Chl-*a* decreased in all trophic levels (all habitats- lake, pelagic and littoral; Table 3). With an increase in water clarity, Zhu et al. (2006) found that after zebra mussel introduction into Oneida Lake, NY macrophyte density and frequency of occurrence increased due to deeper light penetration. The increase in diversity and frequency of occurrence of macrophytes (Zhu et al. 2006) can create more habitats for invertebrates such as Turbellaria and Trichoptera (Aldridge et al. 2004). Similarly, Ricciardi et al. (1997) found

Table 3. Change in abiotic parameters post Dreissena polymorpha invasion of freshwater ecosystems and enclosure experiments (Higgins and VanderZanden 2010). Negative (-) denotes negative change and a positive value denotes a positive change. (Modified from Higgins and VanderZanden 2010.)

	Lake (all habitats)				Pelagic-profundal				Littoral			
Parameter	*n*	%	SE	*P*	*n*	%	SE	*P*	*n*	%	SE	*P*
Secchi	46	38.5	4.7	<0.001	27	30.7	5.5	<0.001	19	50.5	10.7	<0.001
Turbidity	10	-40.7	21.5	0.025	5	-34.7	21.2	0.178	5	-46.2	23.3	0.118
TP	42	-19.5	3.8	<0.001	24	-20.8	4	<0.001	18	-17.9	5.8	0.006
SS	21	-39.7	10.8	<0.001	9	-72.2	8	<0.001	12	-46	10.1	0.001
Chlorophyll-*a*	45	-47.3	6.5	<0.001	26	-37.8	8.1	<0.001	19	-58.1	9.5	<0.001
Littoral depth *									6	39.4	26.4	0.015

* Littoral depth refers to maximum depth colonized by rooted macrophytes

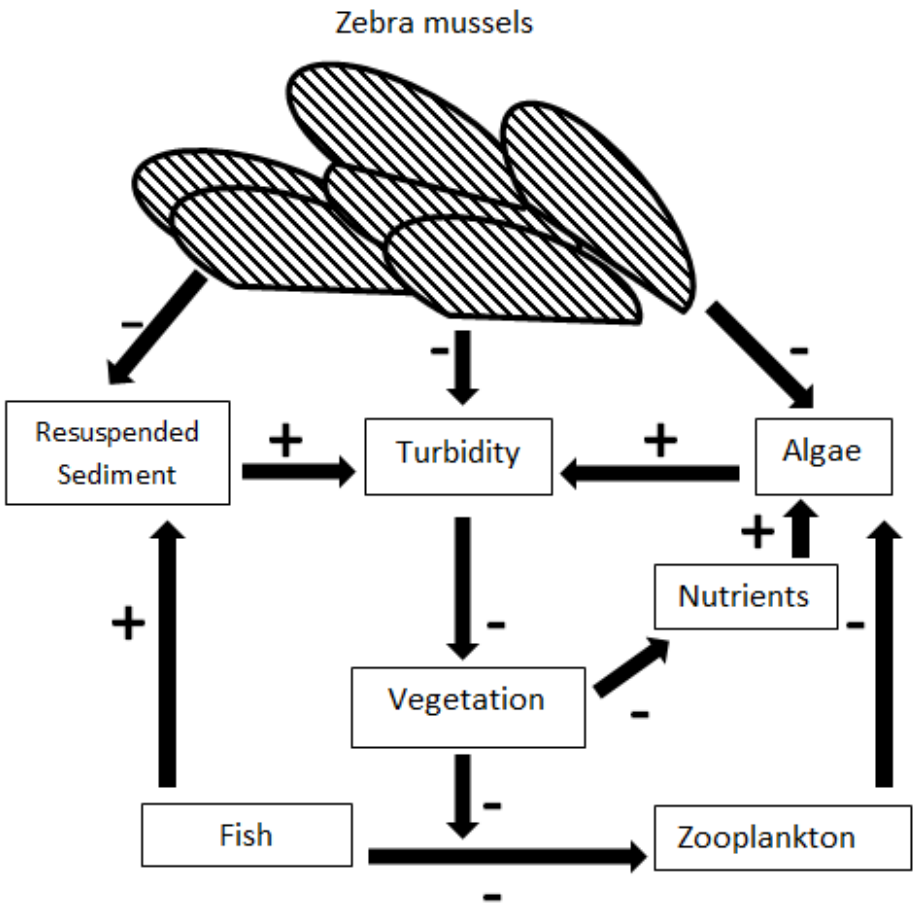

Figure 4. Schematic showing the feedback loop in a reservoir/ lake system. (+) represents a positive correlation and (-) represents a negative effect on the factor. This demonstrates the zebra mussels' ability to change a system from a turbid state to a *clear* state. This is done by suppressing turbidity and re-suspending particles into sediment. Zebra mussels not drawn to scale. (Modified from Scheffer et al. 1993.)

benthic macro-invertebrate community assemblages increased in the presence of zebra mussel, and speculated that some species such as gammarid shrimps may benefit from the increased habitat formed by zebra mussel shells and byssus threads.

It is important to note, however, that although the sequestration of particulates by zebra mussels is an advantage for macrophytes, it may increase competition for resources with other suspension-feeding species (Aldridge et al. 2004). Additionally, although an increase in macrophyte density positively influences zooplankton, macroinvertebrate, and fish diversity, as well as production and food supply; the introduction of other non-native species may have *unanticipated consequences* for the ecosystem (Zhu et al. 2006).

Due to zebra mussels filtration capacity coupled with the ability to reach such high densities, zebra mussels disrupt the normal nutrient cycling within a water body (McLaughlan and Aldridge, 2013). Zebra mussels sequester a large amount of nitrogen and phosphorous by ingesting seston; therefore, these nutrient can no longer be utilized for phytoplankton productivity (McLaughlan and Aldridge, 2013). Zebra mussels can reach densities as high as 750,000 individuals m^{-2}, making up the majority of the benthic biomass in freshwater systems (Kovalak et al. 1993). Consequently, zebra mussels mediate the transfer of particulate nutrients to dissolved chemical forms of nutrients in sediments (Zhu et al. 2006). This shift is noted by Higgins and VanderZanden (2010) demonstrating a greater capacity of zebra mussels to transfer energy pathways from pelagic-profundal to benthic littoral energy pathways, ultimately shifting food web

dynamics to primarily benthic energy. This shift caused by zebra mussels can result in a cascading effect on the biotic environment of the invaded ecosystem (Zhu et al. 2006). Additionally, Aldridge et al. (2004) found that high densities of zebra mussels caused major shifts in the plankton communities of lakes and rivers, and that zebra mussels were a key species in altering all trophic levels (MacIsaac, 1996; Aldridge et al. 2004). Nicholls and Hopkins (1992) reported that since the introduction of zebra mussels to Lake Erie alone, there has been a significant decrease in phytoplankton densities from 5,000 Aerial Standard Units (ASUs) in the late 1960s to less than 1,000 ASUs. Furthermore, these shifts in phytoplankton composition result in a favorable condition for bloom-forming cyanobacteria that can be harmful to humans (Cooke and Kennedy, 2001; McLaughlan and Aldridge, 2013).

Planktonic community changes subsequently influence, aquatic flora and fauna and ultimately disrupts the entire aquatic food web (Aldridge et al. 2004; Zhu et al. 2006; Miehls et al. 2009, McLaughlan and Aldridge 2013). For example, Miehls et al. (2009) found that zebra mussels had the ability to alter Canadian Bay of Quinte's food web by homogenizing the species in the system. After zebra mussel introduction, there was a direct shift from pelagic planktivores to benthic planktivore subgroups, most likely due to a change in energy pathways caused by zebra mussels (Miehls et al. 2009). Although Miehls et al. (2009) found a significant change in the aquatic system they studied, it should be noted that the scope and severity varies among ecosystems. The introduction of zebra mussels into a novel environment exerts long-term effects on

lentic and lotic system (Miehls at al. 2009) by altering the function (productivity and nutrient cycling) and structure (phytoplankton, macrophytes, fish and invertebrate density) of an ecosystem.

Ecological impacts on native mussels

Lastly, Strayer (1999) noted one of the most dramatic ecological effects caused by zebra mussels is the influence exerted onto local native Unionid mussel populations that has led to substantial population declines. Native mussels are important to the ecosystem as bioindicators and as a food source for many biota (Guevara et al. 2004). Unfortunately, zebra mussels have nearly extirpated native unionid clams from infested waters by fouling (as a *biofouler*) their shells, increasing costs of locomotion, interfering with normal valve movement, deforming the valve margins and outcompeting for food (Strayer, 1999; Aldridge et al. 2004; Nicholls and Hopkins, 1992). Studies have shown that zebra mussels biofoul native mussels causing starvation, energy reserve depletion, and the inhalation of metabolic waste created by the zebra mussel causes death in native mussels (Strayer, 1999). As zebra mussels in high densities filter the water column more readily often outcompeting their native bivalve relatives for food (Nicholls and Hopkins, 1992).

The native range of zebra mussels is situated in the western portion of the Palearctic Region, a term to specify origin of terrestrial/ aquatic fauna (Brown and Lomolino, 1998). Graf (2007) indicated 45 biological species of freshwater mussels that are native to the Palearctic Region. Of all the mussels identified, the majority were the

thick shelled river mussel (*Unio crassus)* and duck mussel (*Anodonta* spp.; Graf 2007). In the zebra mussel's native range, there is less mussel diversity than in the rivers running through them (Kentor et al. 2010). Other mussels potentially found in the zebra mussels native range include, the swollen river mussels (*Tumidiana tumida*), painter's mussel (*Unio pictorum*), Mediterranean/ black mussel (*Mytilus galloprivincialis*), sand mussel (*Chamelea gallina*) and many other *Dreissena* spp. These freshwater mussels may be able to survive zebra mussel biofouling behavior due to higher predation from aquatic fauna (*i.e.*, Roach) that may not be present in North America, although no literature was found that directly addressed this topic.

In North America, there are minimal fish predators that will eat zebra mussels, and therefore, freshwater mussels are in direct competition with zebra mussels and major declines are being seen worldwide (Strayer, 1999; Aldridge et al. 2004). Moreover, without evolving with a fouling organism like zebra mussels, North American Unionid mussels are at a disadvantage in many ways and do not possess adaptive mechanisms to mitigate their effects (Ricciardi, 2003). As a result, the North American native freshwater mussel rate of extinction has accelerated by 10-fold (Ricciardi and Rasmussen, 1999). For example, the extinction of the threeridge mussel (*Amblema plicata*) will occur within the next 50 years if survival rates in the presence of zebra mussels stays consistent (Hart et al. 2004); however, it is important to note, each unionid species has a unique zebra mussel sensitivity (Strayer, 1999).

As with any animal population, understanding population dynamics and habitat preferences are critical to management strategy development. Interestingly, the Alexandria area of Minnesota is unique in the fact that there is anecdotal evidence indicating historic native mussel populations and possible extant populations. This study was done in four out of the eleven Alexandria chain or lakes and will address zebra mussel negative phototoxic behavior quantified by density, as it relates to biomass of vegetation. Unpublished studies on macrophytes as zebra mussel substrate, includes an undergraduate team from the UM who looked at Eurasian watermilfoil (*Myriophyllum spicatum*), clasping-leaf pondweed (*Potamogeton richardsonii*) and coontail (*Ceratophyllum demersum*) as potential substrates among several bays in Lake Minnetonka, Hennepin and Carver County, MN.

The second non-peer reviewed study addressed the habitat suitability of zebra mussels in different areas of Lake Minnetonka but minimal was done in testing zebra mussels preference to aquatic macrophytes The researchers suggested in their future research section (Salverson and Zelickson, 2015) that there is need to further investigate zebra mussel macrophyte preference. Only a few publications address the question of zebra mussel affinity for vegetation, and those studies were done in the zebra mussels' European invaded ranges (Lewandowski and Ozimek, 1997; Ozimek 1997; Muskó and Bako, 2005). I was not able to locate any published studies in the United States. Furthermore, few studies have addressed macrophyte and zebra mussel habitat relationships and this study will add information to a literature gap. It will also

facilitate questions regarding native mussel populations in this system and allow for similar inferences to be made regarding zebra mussel populations in area lakes of Minnesota by assessing habitat preferences.

Methods and Study Area

Study Area

The lakes in and near Alexandria, Minnesota were considered for research in part because it is one of only a few identified areas where zebra mussels and native mussels are cohabitating. This chain-of-lakes system is highly populated by recreationalists in the summer months, and offers an area for aquatic recreational activities. Managing for zebra mussels in this area should be a high priority for the overall economic well-being of this city.

Paternoster lake (*e.g.,* the Alexandria chain) directly translated to *String of Beads* are lakes that form as glaciers recede and form connected bodies of water via channels or streams (Umesh et al. 2011). A chain-of-lakes system is usually connected via a channel or river system ultimately adding more variability to the system rather than what would be present in a single-lake system. The study area had historic glacial deposits from the Des Moines lobe of the Laurentide Ice Sheet, ultimately lending to the hydrological connectivity in the study lakes (MN DNR, 2015).

Furthermore, this connectivity allows for movement of biota among water bodies. Water is always flowing from the headwaters to the mouth, and moving throughout the system; therefore, often sharing similar species and geologic formation. Soranno et al. (1999) states, “Neighboring lakes sharing a common climate, geologic setting, and regional species pool differ systematically in many of their features as a function of their hydrology and geomorphology”. Minimal literature publications have actually addressed differences between endorheic (closed drainage basin with no

outlets) and exorheic (open drainage basin with outlets) systems and their biology. Additionally, although similarities are seen among hydrologically linked lakes, it is unclear how this may affect zebra mussel density. Thus, this research will give insight into hydrologically similar paternoster systems and linked geological habitat for zebra mussel assemblages.

Alexandria, Minnesota is a popular tourist town situated in the west-central part of Minnesota with a population of 11,070 (U.S. Census Bureau). The town is part of Douglas County and the lakes are all part of the Upper Mississippi River major drainage basin and the Long Prairie major watershed (Figure 5). The Alexandria lakes area has been a place of vacationing since the 1800s when Midwesterners traveled by train to fish, lodge and enjoy everything this town has to offer (Lakes' Area Chamber of Commerce 2014). Four of the eleven lakes that make up the Alexandria Chain-of-Lakes were sampled as indicated by the bathymetric and topographic maps (Figure 6).

Land type for the land surrounding all four lake are Eastern Broadleaf Forest Province and geomorphology is all rolling to gently rolling terrain such as hills or ridges from the supraglacial drift complex. Soils surrounding all four lakes are prairie soils with a mean temperature cooler than 47°F. Topography shows minimal areas with high elevation being at the North-west portion of Lake Carlos and a small portion on the south-west and south-east part of Lake Geneva (Figure 6). The primary land use for the study site includes open water (25%), cultivated crops (17%), developed, open space

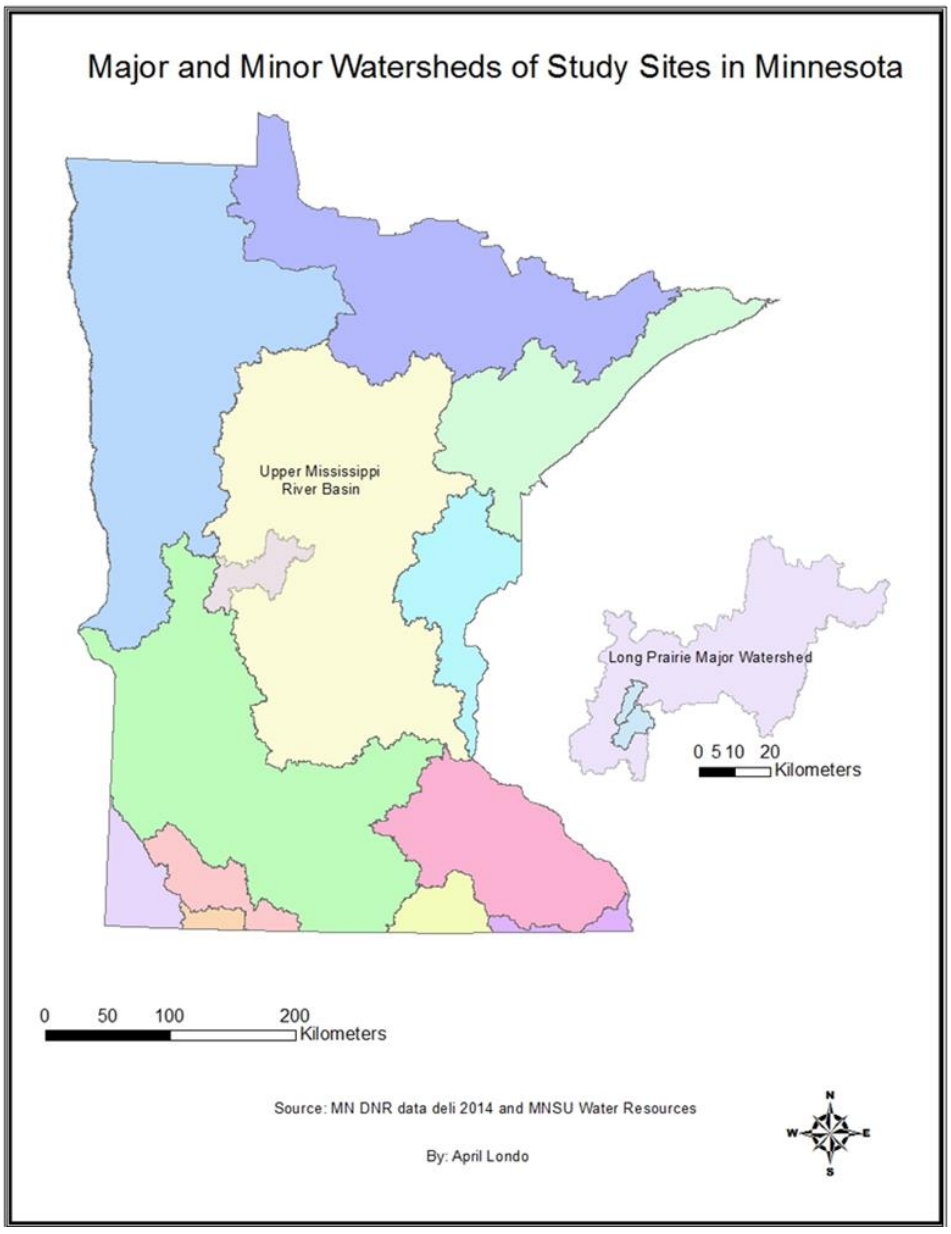

Figure 5. Long Prairie Major Watershed within the Upper Mississippi River Basin. Study sites are within these boundaries. Data gathered from the MN DNR data deli and MNSU Water Resources.

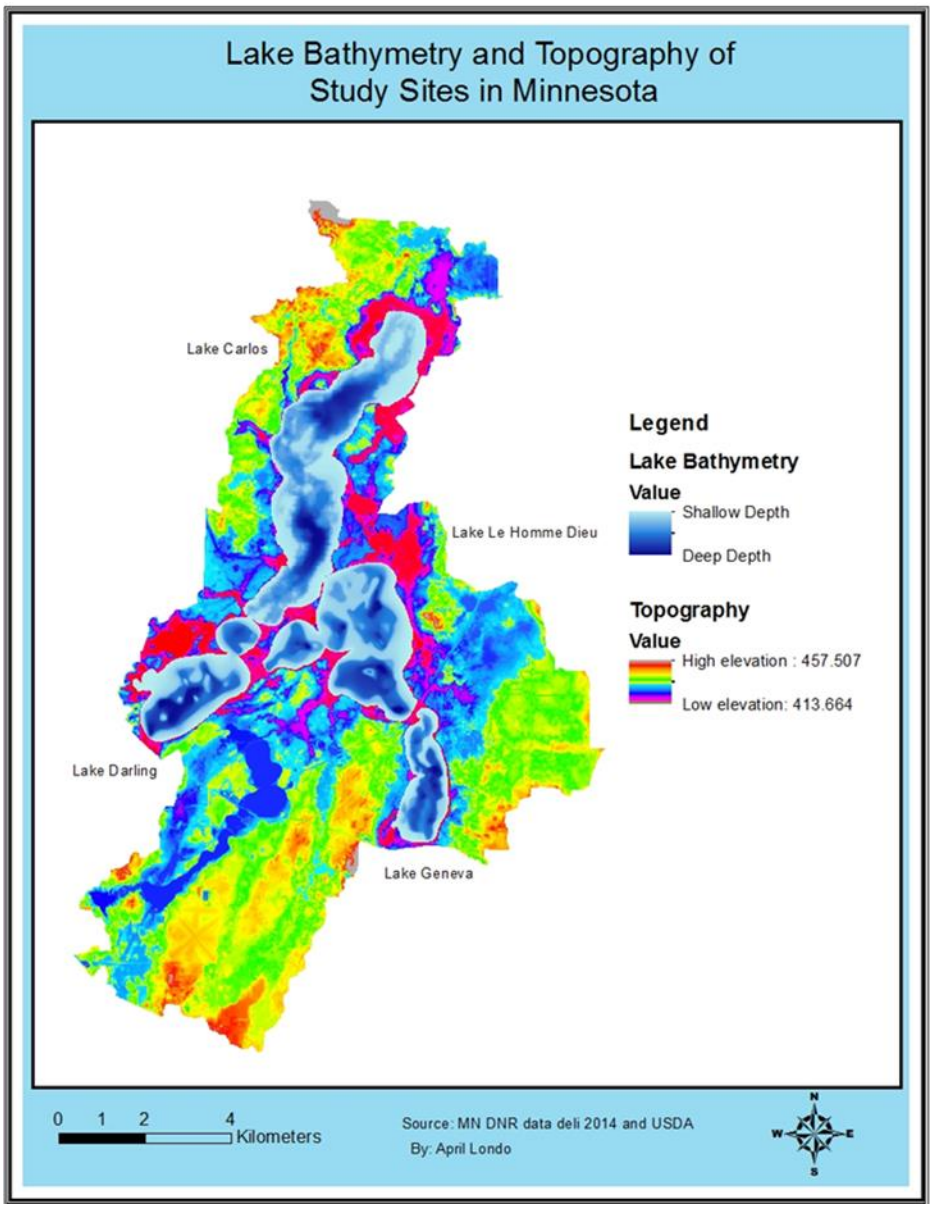

Figure 6. Four of the Eleven Lake that make up the Alexandria Chain of Lakes. Lake Carlos, Lake Le Homme Dieu, Lake Geneva and Lake Darling. Topographic and bathymetric images obtained using MN DNR data deli and USDA. Darker blue represents deeper area for lake bathymetry. Higher elevation are depicted as red and lower elevation as magenta.

(14%), deciduous forest (12%) and hay/ pasture (11%). The remainder of land is made up of herbaceous (5%), developed, low intensity (4%), developed, medium intensity (4%), emergent herbaceous wetlands (2%) and developed, high intensity (1%) (Figure 7).

Lake Carlos is located 17.2 km northeast of the center of Alexandria, MN and has one public access located in the Lake Carlos State Park on the northeast most portion of the lake (Figure 8). Lake Carlos and Lake Darling are in the Lake Carlos minor watershed that is 17,629 acres (7134 ha) in size. The total lake area is 2,605 acres (1,054 ha) and is the biggest of the lakes being sampled. The littoral area and the maximum depth is 922 acres (373 ha) and 49.6 meters respectively (MN DNR 2014). Lake Carlos is connected to Lake Le Homme Dieu via a canal on the southeast corner of the lake and is connected to Lake Darling at the southwest corner of the lake.

Lake Le Homme Dieu (Figure 8) is the second largest lake sample with a lake area of 1,801 acres (728 ha) and a maximum depth of 25.9 meters. Lake Le Homme Dieu and Lake Geneva are situated in the Lake Le Homme Dieu minor watershed which is a total of 10,320 acres (4,176 ha) in size. It is situated just 11.7 km northwest of Alexandria, MN. The littoral area of Le Homme Dieu covers 767 acres (310 ha) (MN DNR 2014). Lake Le Homme Dieu is connected to Lake Carlos at the northwest portion of the lake and is connected to Lake Geneva at the northeast corner of the Lake. Lake Darling is 6.6 km west of the center of Alexandria, MN and is third largest of the lakes being sampled, at 1,050 acres (425 ha) (Figure 8). The littoral area encompasses 477 acres (193 ha) and has a maximum depth of 18.9 m (MN DNR 2014).

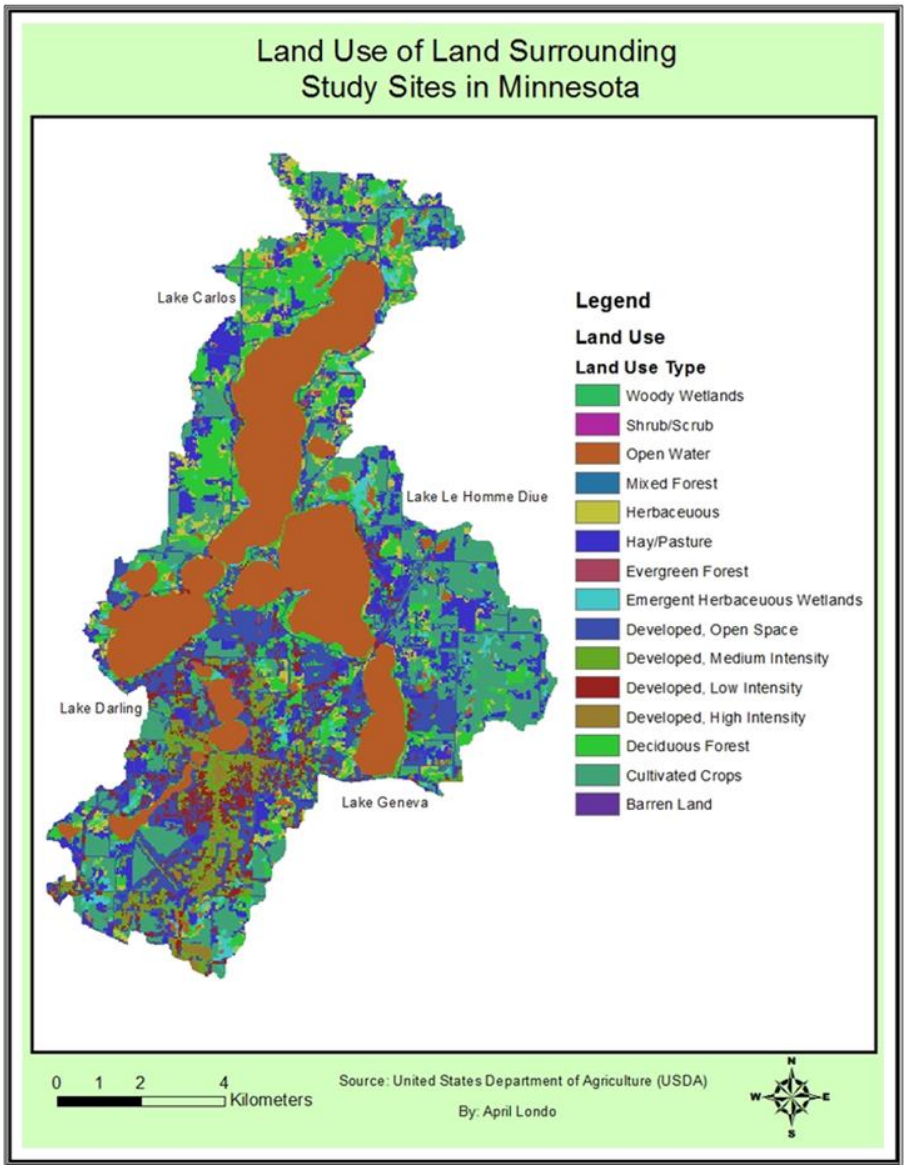

Figure 7. Land use for the study area, boundary around lakes are the Lake Le Homme Dieu and Lake Carlos minor watersheds. Data obtained via United States Department of Agriculture. Colored areas represent land use parameters.

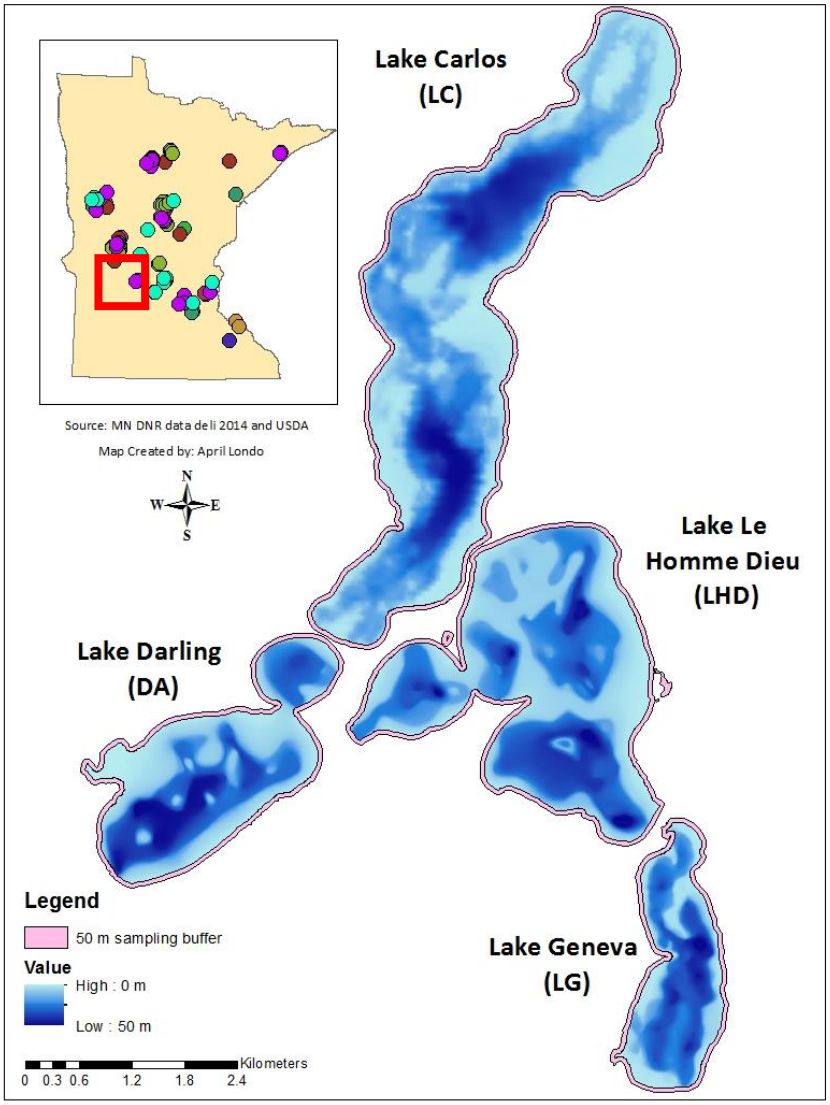

Figure 8. Four bathymetric maps of Lake Carlos, Lake Le Homme Dieu, Lake Darling and Lake Geneva, all in northern MN. Topographic and bathymetric images obtained using MN DNR data deli and USDA. Darker blue represents deeper area for lake bathymetry. Lakes were sampled in July 2015.

There is no public boat access on this lake therefore it was sampled by entering the water at the public boat access on Lake Le Homme Dieu and Lake Carlos will be by-passed to get to the study sites. Lake Darling is connected to Lake Carlos at the upper northeast portion of the lake and at the southwest portion connected to Lake Cowdry (not in study).

Lake Geneva (Figure 8) has an area of 640 acres (259 ha) and a littoral area of 265 acres (107.24 ha) (MN DNR, 2014). It has one public boat landing on the northwest side of the lake, situated close to the connection point into Lake Le Homme Dieu. It has a maximum depth of 19.2 meters (MN DNR, 2014) and is also connected to Lake Victoria (not in study) to the south. It is 6.28 km from Alexandria, MN.

Field Design and Data Collection

Self-contained underwater breathing apparatus (SCUBA) was utilized from a boat at each lake to obtain physical samples and observational data. The required sampling gear included a regulator, Buoyancy Control Device (BCD), tanks, wetsuit, mask, fins, snorkel, transect line (meter tape), 0.25 m^2 quadrat frame, and sample containers. Field notes, including weather and lake conditions were recorded at the time of data collections.

Lake transect allocation was done by systematic random sampling. Using bathymetric maps as template (Figure 8) transects were selected by overlaying three randomly selected diameter lines run through the center point of each lake that extended to the shore line in each direction. One line was drawn directly through the

lake running North and South, one from North-East to South-West and the last was drawn South-East to North-West. Transects were positioned at each of the six points the diameter lines intersected with the shoreline (Figure 9).

The 50-m transect line was then secured by the diver where the water met the terrestrial organic biomass, natural and/or man-made barriers at the periphery of the lake. GPS coordinates were taken by the assistant on the boat deck where the diver secured each transect line (Table 4). The diver swam the meter tape perpendicular to the shore directly outwards towards the center of the lake. When the tape reached 50 meters the diver swam the tape down and situated a buoy onto it to denote the end of the transect. Usually depth intervals, not distance intervals, on the transect line are sampled (Joiner 2001), however, due to the minimal depth gradient at each lake, distance intervals were used (Nadaffi et al. 2010).

The 0.25 m^2 quadrat used for sampling was comprised of a metal frame and a mesh bag (6.35-mm mesh) situated at one end (Figure 10). Placement of the quadrat was done by hovering over the transect depth interval and dropping the quadrat to the right of the transect line looking at shore. The sample was taken where the quadrat landed. This was done at five 10-m intervals along the transect line, with the first interval 50 m from the shoreline. The diver then descended to the 50-m position facing towards shore to collect the first sample. The diver then proceeded to descend to the quadrat to collect the contents (plant and zebra mussel specimens) confined within. This

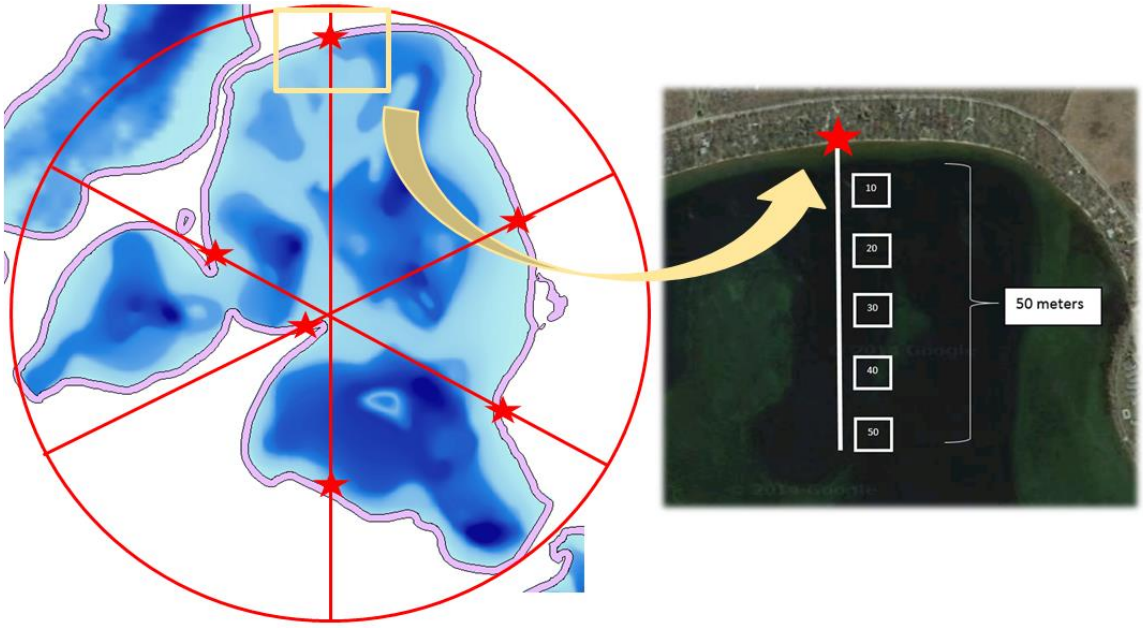

Figure 9. Transect allocation in each study lake. Red star indicates the transect point intercept. A 50 meter transect line was laid perpendicular to shore. Five quadrats were placed in 10 m intervals along the transect line.

Table 4. GPS location where transects were places at each lake (Darling, Geneva, Le Homme Dieu and Carlos). Six transects were positioned perpendicular to the lakes' edge. They were placed in July, 2015.

	Lake GPS Locations			
Transect	Carlos	Darling	Geneva	Le Homme Dieu
1	N 45° 59.269 W 95°20.470	N 45° 55.889 W 95° 22.748	N 45° 54.732 W 95° 19.448	N 45° 55.014 W 95° 19.897
2	N 45° 58.465 W 95° 21.476	N 45° 55.359 W 95° 22.948	N 45° 54.318 W 95° 19.359	N 45° 55.940 W 95° 21.633
3	N 45° 56.195 W 95° 22.376	N 45° 55.419 W 95° 24.114	N 45° 53.731 W 95° 19.212	N 45° 55.854 W 95° 32.211
4	N 45° 57,218 W 95° 21.158	N 45° 54.815 W 95° 24.905	N 45° 53.329 W 95° 20.033	N 45° 55.528 W 95° 21.116
5	N 45° 58.293 W 95° 21.030	N 45° 55.436 W 95° 23.896	N 45° 53.914 W 95° 19.923	N 45° 55.006 W 95° 21.047
6	N 45° 59.436 W 95° 19.573	N 45° 55.221 W 95° 22.818	N 45° 54.286 W 95° 19.956	N 45° 54.753 W 95° 20.312

Figure 10. Quadrat used for sampling in study lakes. Consisted of a 0.25 m^2 metal frame with a bag at one end for collecting specimens.

was done by digging into the sediment 7 to 8 cm to uproot plants and collect all zebra mussels within the quadrat. Notes were taken on a dive slate including depth, substrate (% per substrate type classification), plant taxa, vegetative cover (%), and additional observations. How much of the quadrat contained macrophytes was recorded as vegetative cover. Substrate classification type(s) was visually estimated as proportions using an adaptive Wentworth scale (Table 5; Wentworth, 1922). The substrate proportions were later used for analysis. Additional information regarding bottom substrate was observed including the presence of detritus (*i.e.*, leaf litter, smaller pieces of wood, and other organic allochthonous materiel), logs, garbage, and organic biomass in each quadrat.

A dive rope was fastened to the quadrat via a karabiner which was then securely fastened to the boat. After the specimens were collected and notes were taken the diver ascended. The quadrat was then pulled up by an assistant on the boat deck by the dive rope, and each quadrat was put into a container, marked internally and externally with the sample number [lake abbreviation (Lake Carlos-LC), transect number (1), quadrat number (50 m) *i.e.*, LC150]. The samples were stored separately, returned to the laboratory, and frozen at -18° C (0°F) for subsequent analyses. This process was done to each quadrat at each transect line at each lake for a total of 30 samples per lake.

Table 5. Classification of substrate types based on the range in particle size diameters. Modified using Wentworth scale of rock particle size (Wentworth, 1922).

Substrate type (classification)	Particle Size in diameter (mm)
Clay	<1/256 (0.004)
Silt	0.004-0.06
Very Fine Sand	0.13-0.25
Fine Sand	0.125-0.25
Medium Sand	0.25-0.5
Coarse Sand	0.5-1
Very Coarse Sand	1-2
Gravel	2-4
Pebble	4-64
Cobble	64-256
Boulder	>256

1862; Skawinski 2014) and state agency staff verification. In the context of this study, *organic biomass* shall refer to anything that is not geological substrate, including submerged and emergent macrophytes, algae, macroalgae, and detrital materials. Native mussels were seen as substrates but were assessed separately. Organic biomass and density of zebra mussels attached to organic biomass were documented.

Zebra mussels were separated from each type of organic biomass and counted to achieve a population count and size structure per organic biomass type. Collection was done by obtaining a piece of organic biomass and starting at the top of the plant and continuing to the base of the plant, picking each zebra mussel off with a forceps. Resistance felt when pulling on the zebra mussel corresponded to byssal attachment. Only living zebra mussels can attach to organic biomass, therefore all dead mussels found on organic biomass was added to the free dead zebra mussel density. Up to the first 50 zebra mussels found on each organic biomass type were saved in a collection jar and frozen for later measurement or were measured that day. The remaining mussels that were not selected on each organic biomass type were enumerated and recorded as density, calculated as density per organic biomass. The selected zebra mussels were measured by using a Vernier caliper.

To determine organic biomass (g dry weight per 0.25 m^2), dry weights were collected from all organic biomass found excluding unionids and synthetic objects. After all zebra mussels were separated from the organic biomass, each taxa was blotted dry with blotting paper. Petri dishes were weighed before organic biomass was added and

Laboratory Assessment

A total of 120 samples were processed in the laboratory. Samples were thawed and materials separated, to the greatest extent possible, into groupings of macrophytes, algae, detrital material, and native mussels. For the purpose of this study, *free* mussels were mussels detached from organic biomass and were at one point attached to geologic substrate. The zebra mussels left in the strainer were assumed to be mussels that were attached to the geological substrate observed in each quadrat. After separation, the *free* mussels were strained through a 500-micron sieve to filter additional sand and silt, but retain mussels as small as 0.9 mm.

Zebra Mussels Categorized as "Free"

The mussels left in the strainer were analyzed as free mussels, were enumerated for density calculations and length measurements were taken for size structure. Mussels were then transferred to a holding tray and the strainer contents were rinsed three times to remove all mussels contained in the strainer. Differentiating between living and dead mussels was done by observation. Mussels were considered alive if they still contained soft tissue at the time of analysis. Soft tissue indicated the mussel was alive before the sample was frozen. Dead mussels did not contain soft tissue, and therefore were counted as whole and half shells. Both live and dead mussels were analyzed separately for each sample.

Fifty live and fifty dead zebra mussels were randomly selected and saved from each sample for length measurements. This zebra mussel selection process occurred by using a transparency grid consisting of twenty 7.26 cm^2 squares numbered 1 to 20 to

obtain length measurements. The selection grid was placed underneath a clear sorting pan of the same size that contained the free strained mussels (both living and dead). Numbers were selected using a random number generator. The selected number space in the pan was then enumerated of all mussels. This continued until 50 live and 50 dead mussel lengths were recorded. The selected free mussels were then put into a collection jar with sample number, date, initials of the person who processed the sample, and then frozen. The remaining mussels that were not selected were enumerated and recorded as living and dead zebra mussel density.

Length measurements were done by using an 8 x 11.5 in glass pan. Collection jars were thawed and water was put into jar, swirled and contents were poured into glass pan. The collection jar was rinsed three times to make sure all mussels were available for measurements. Zebra mussel lengths were measured using a Vernier caliper to the nearest 0.1 mm from the umbo to the dorsal margin of the shell (Muskó and Bakó 200). Fifty live and 50 dead zebra mussels were measure for length to achieve a size structure. If the sample did not contain 50 of each, all observed zebra mussels were enumerated and measured.

Zebra mussels attached to Organic biomass

Separated macrophytes, algae, and detrital material were identified upon thawing. Identification was accomplished using a combination of text resources (Carol 1862; Skawinski 2014) and state agency staff verification. In the context of this study, "organic biomass" shall refer to anything that is not geological substrate, including

submerged and emergent macrophytes, algae, macroalgae, and detrital materials. Native mussels were seen as substrates but were assessed separately. Organic biomass and density of zebra mussels attached to organic biomass were documented.

Zebra mussels were separated from each type of organic biomass and counted to achieve a population count and size structure per organic biomass type. Collection was done by obtaining a piece of organic biomass and starting at the top of the plant and continuing to the base of the plant, picking each zebra mussel off with a forceps. Resistance felt when pulling on the zebra mussel corresponded to byssal attachment. Only living zebra mussels can attach to organic biomass, therefore all dead mussels found on organic biomass was added to the free dead zebra mussel density. Up to the first 50 zebra mussels found on each organic biomass type were saved in a collection jar and frozen for later measurement or were measured that day. The remaining mussels that were not selected on each organic biomass type were enumerated and recorded as density, calculated as density per organic biomass. The selected zebra mussels were measured by using a Vernier caliper.

To determine organic biomass (g dry weight per 0.25 m^2), dry weights were collected from all organic biomass found excluding unionids and synthetic objects. After all zebra mussels were separated from the organic biomass, each taxa was blotted dry with blotting paper. Petri dishes were weighed before organic biomass was added and the weight, sample number and date were written on the side of the petri dish. Blotted organic biomass were added to each petri dish making sure that each dish consisted of a

different taxa. Each taxa was dried and weighed separately of one another. Using previously described organic biomass drying procedures, samples were dried for a minimum of 96 hours and checked in 48-hr intervals at 105˚C until no net water loss could be recorded and a constant weight within 0.03 grams was achieved (Gross et al. 2001; Newman and Biesboer 2000). End weight was recorded and the difference was calculated for biomass.

Zebra mussel density was also enumerated from every native mussel shell observed in each sample. This was done by scarping off zebra mussels from the native mussel carapace into a glass dish. Water was added to the glass dish and the transparency grid was used to randomly select 50 live and 50 dead zebra mussels. Dead zebra mussels were recorded in this substrate type because byssal clusters will form over both dead and live zebra mussels. Numbers were selected using a random number generator. The selected zebra mussels were measured while the remaining were enumerated for total density per native mussel taxa. Total carapace length was taken from each whole native mussel by using Vernier calipers, while pieces of native mussel shells were counted.

Data Analyses

Zebra mussel densities (#/m^2) were assessed among lakes dependent on substrates (both geologic and organic). Shapiro Wilks was used to determine if any significant differences were present. If the data were normally distributed, an analysis of variance (ANOVA) among densities of each lake was completed. If the data were not

normally distributed, data were log-transformed. A log(n+1) transformation was used to normalize zero data. If the transformed data were normally distributed an ANOVA was used. If the data set still fails normality, a nonparametric Kruskal-Wallis test was run. After running an ANOVA the data were tested for significant differences among lakes. If those data did not show significant differences ($P<0.05$), all four of the lakes have shown to be similar and the N value is 120.

If there was a significant difference among lakes after the ANOVA is run a Tukey's test was run to identify where the differences existed. If there was a significant difference among the lakes after running the non-parametric test, a Dunn's test was run to identify where the differences between lakes exited. In both cases the N value was 4. Analysis of variance was run among lakes in comparing total zebra mussel density, depth (m), total organic biomass (g/m^2 dry weight), zebra mussel density (#/unit) per organic biomass, juvenile zebra mussel density per organic biomass and, adult zebra mussel density (number) per organic biomass, and vegetative cover. If data is not normal a Kruskal-Wallis was run on ranks.

Geologic substrates phi (ϕ) values were calculated from field observations. In order to normalize the percentages of geologic substrate per quadrat, a phi value (ϕ) was calculated as

phi value (ϕ) = $[-\text{Log}_2(\text{sediment size, mm})\cdot(\text{percentage, \%})]$.

The phi calculations were completed by skewing quantified percentages using the sediment grain-size distributions based on the Wentworth sediment classification scheme [*i.e.*, sand (2 mm to 63 μm, −1 to 4ϕ), silt (31.5–4 μm, 5–8ϕ) and clay (< 2 μm, < 9ϕ); Berkman et al 2000]. A multiple regression was run for the 120 quadrats with zebra mussel density as the dependent variable on depth, substrate phi (ϕ) values, organic biomass, and vegetative cover. Normality was tested using a Shapiro-Wilk test. When data met normality, an ANOVA was run to determine if significant differences were present among depth categories. If data was not normal, a Kruskal-Wallis was run on ranks. In both cases, when a significant difference was detected, multiple comparisons were completed to identify where those differences were located.

Organic biomass categories and zebra mussel densities among the organic biomass categories were each tested for normality. If normally distributed, an ANOVA was used to compare zebra mussel density (number) per organic biomass (g dry weight). If normality was not met, a Kruskal-Wallis was run on all taxon. Only those organic biomass categories that made up at least 1% of the total biomass were included in the analyses. If filamentous algae and *Chara* spp. were not significantly different from each other, they were collectively advanced in the assessment as “algae.”

To evaluate zebra mussel preference among the organic biomass categories, a linear electivity index (LEI) was utilized (Strauss 1979). The LEI allows for the determination of the degree of electivity:

$$LEI = r_i - p_i,$$

where r_i is the relative abundance of organic biomass category "i" with zebra mussels present and p_i is the relative abundance of organic biomass category "i" in the environment. Output values for the LEI range from 1 (strong electivity) to -1 (complete avoidance). Therefore, a LEI value of 0 suggests zebra mussels are using the category at a rate proportional to its presence in the environment.

Adult and juvenile zebra mussel attachment to organic biomass was assessed. Based on the overall ratio of adult and juvenile zebra mussels as a whole, the expected frequencies of each was applied to each organic biomass category. A chi-square test was then used to run to determine if significant difference between expected and observed frequencies in the proportions of adults and juvenile zebra mussels was present. Mean adult and juvenile densities were also tested for normality, and evaluated with an ANOVA to determine if a significant difference among adults and juveniles among lakes and organic biomass categories was present. If data were not normally distributed, a Kruskal-Wallis was run on ranks. A t-test was done to determine if there were significant differences between juvenile and adults for each category.

Lastly, a regression was run between native mussels' lengths and average zebra mussel length attached to the mussels. Chinese mystery snail (*Cipangopaludina chinensis)*, another mollusc found in the study lakes was also analyzed. A t-test was run between the length and number of zebra mussel attached to native mussel in comparison to length and number of zebra mussels attached to Chinese mystery snails.

Results

Collective Lake Comparison

Objective 1

A total of 157,286 zebra mussels were collected among the 118 correctly processed 0.25-m^2 quadrats evaluated in the four study lakes combined. The mean zebra mussel density, for all sites combined, was therefore 5,331/m^2 (SE=425) and 98% of all quadrats contained zebra mussels. There were no significant differences in zebra mussel densities among the four lakes sampled (Figure 11); however differences among individual lakes were present (see Individual Lake Evaluations section below).

The majority (73%) of all the quadrats sampled had a total phi (ϕ) value of 0 to 1, indicating small particulate size for zebra mussel attachment. When analyzing all four lakes, there was no statistically significant differences among phi (ϕ) values and density of zebra mussels (P= 0.054; Figure 12). Lake Darling was significantly shallower (median=0.427 m; P<0.05) than the other study lake medians (Figure 13a). Lake Darling was the only lake consisting of depth greater than 3.35 m, however, those data points were deemed to be outliers and subsequent analyses were completed with and without inclusion of those points. Samples from Lake Le Homme Dieu had minimal variation in depth (0.9 to 2.1 m), whereas, Lake Darling had that greatest depth range (0.4 to 8.5 m). For all quadrats, zebra mussel densities were significantly different between the 0.7-0.8 m (median=160) and the 2.1-2.2 m (median=7,690) depth intervals (P<0.05), but were not significantly different (P>0.05) among all other depth interval pairings (Figure 13b).

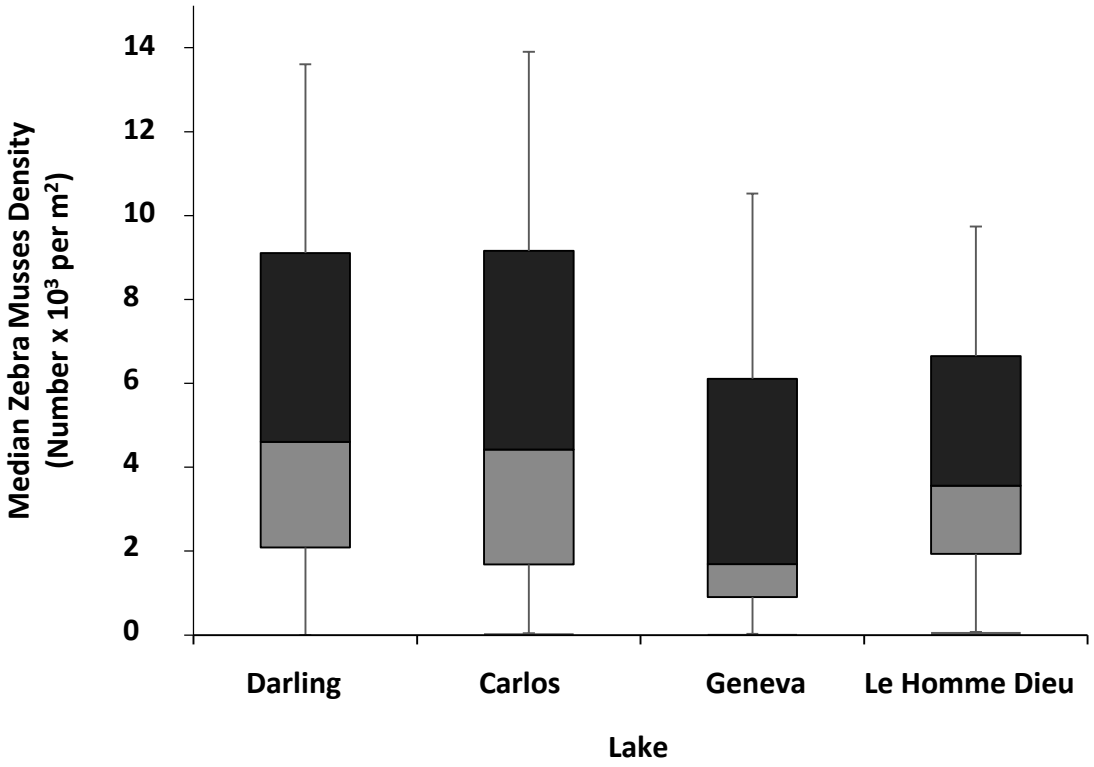

Figure 11. Zebra mussel densities (number x 10^3 per m^2) for four infested lakes in west-central Minnesota in the summer of 2015. The box plots represent the median, 25^{th} and 75^{th} percentiles, and the range of data (vertical bars). Densities were estimated based on observations from thirty 0.25-m quadrats from each lake, thus N=120. No significant differences were present among lakes (P>0.05).

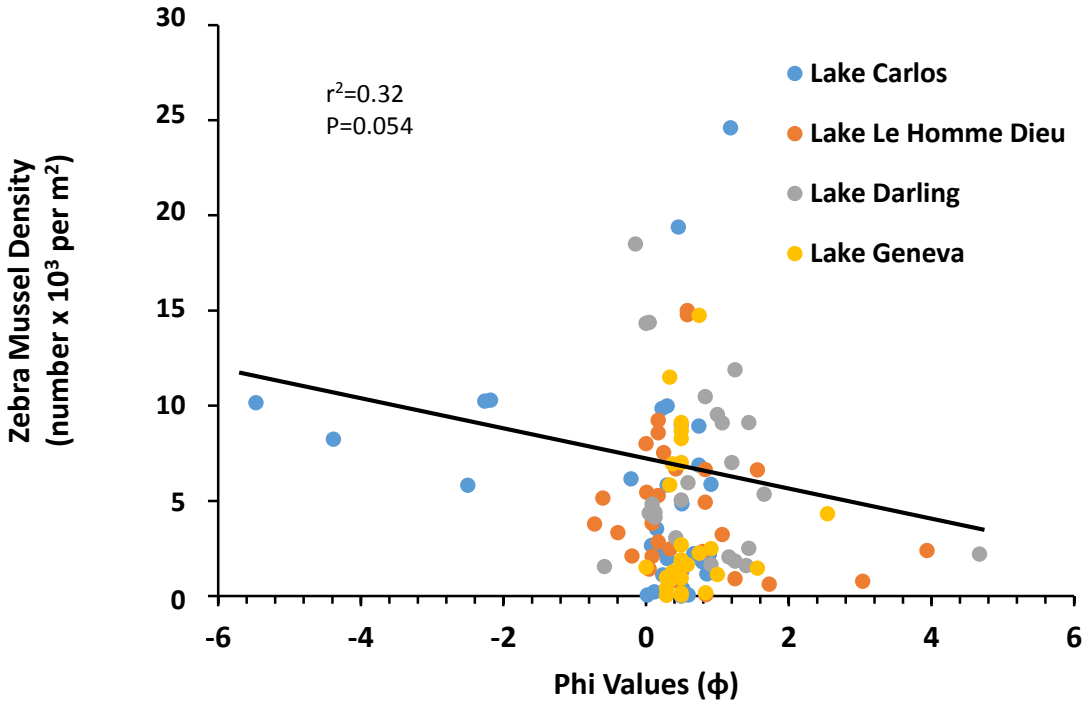

Figure 12. Zebra mussel density (number x 10^3 per m^2) as a function of phi value (ϕ) for four west-central Minnesota lakes in the summer of 2015. Negative phi values indicate larger substrates such as boulders and gravel. Positive phi values indicate smaller substrates such as, sand, silts, and clays. Blue, orange, grey, and yellow dots represent data pairings from lakes Carlos, Le Homme Dieu, Darling, and Geneva, respectively. The r^2 and P-values are denoted. N=30 for each lake.

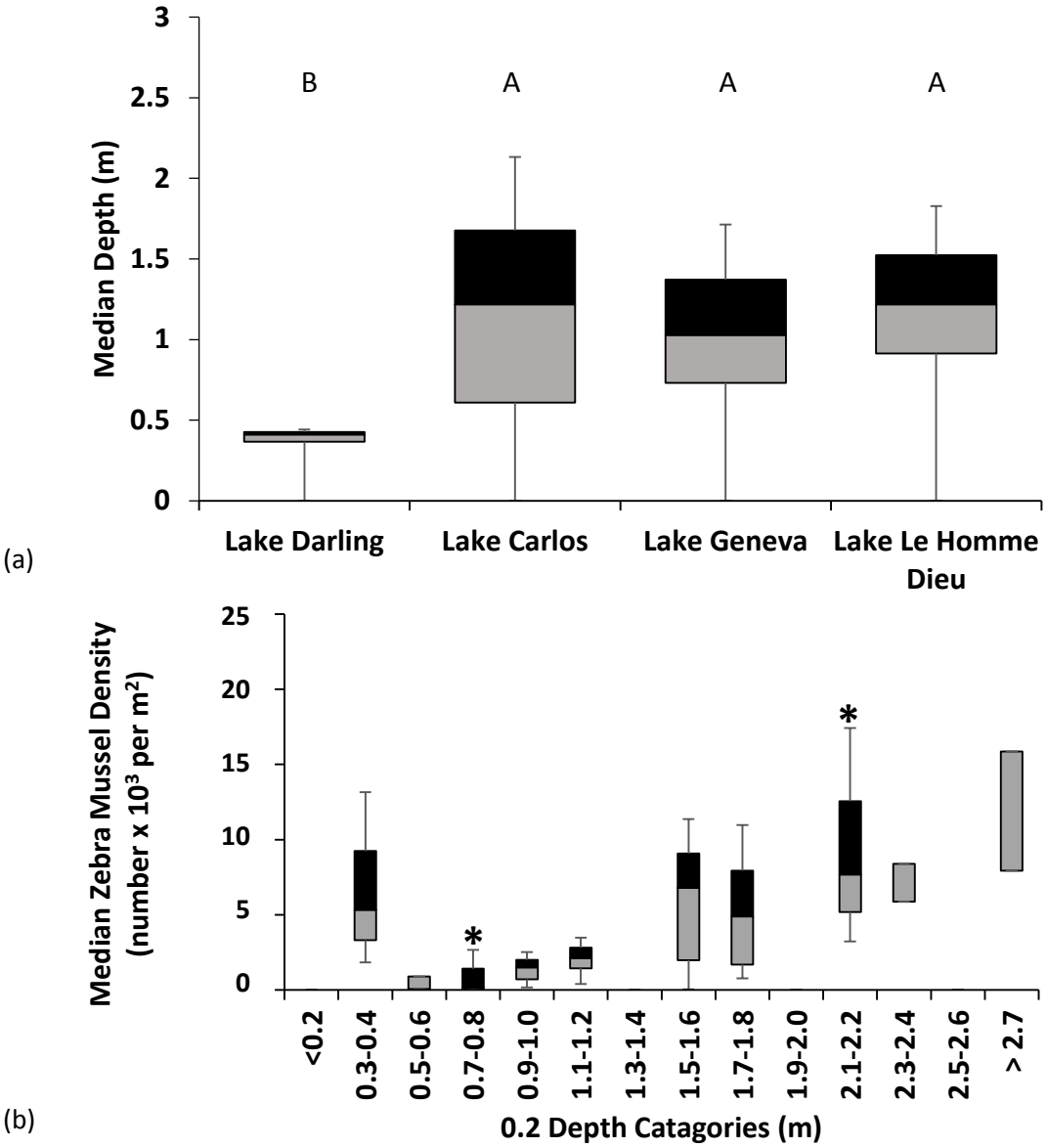

Figure 13. Median depth (m; 11a top figure) and zebra mussel densities (Number x $10^3/m^2$; 11b bottom figure) within 0.2-m depth intervals from a chain of lakes in west-central Minnesota (N=120) in the summer of 2015. Lake Darling was significantly more shallow than the three other lakes ($P<0.05$). Significant differences in 11a are designated by different letters. Zebra mussel densities were not significantly different ($P>0.05$) among depth intervals except for a significant difference between the 0.7-0.8 m and 2.1-2.2 m intervals ($P<0.05$). The significantly different intervals are designated by the asterisks (*).

In the regression between depth and density of zebra mussels among lakes, there was a positive correlation when after the two outliers (from Lake Darling) at 8.5 and 6.4 m were removed (P=0.0004; Figure 14).

Objective 2

Total organic biomass for all quadrats combined was 31,416 g/m^2dry weight. Of that total, 9,709 g (31%) was from Lake Geneva. Lakes Carlos, Darling and Le Homme Dieu were comparable in proportional biomass found, with 7,910 (25%), 7,661 (24%), and 6,229 (20%), respectively. Four out of 120 quadrats sampled were void of organic biomass and 3 of those quadrats were in Lake Darling. *Chara* spp. was the greatest single-category proportion of the biomass (6,160 g). *Ruppia* spp. grasses and white water lily (*Nymphaea odorata*) had the lowest proportions of the biomass (1 g each) and were the only plants void of zebra mussels.

Habitat assessments revealed no significant differences (P>0.05) in median overall organic biomass among the four study lakes. Zebra mussel density was not statistically correlated with biomass (P=0.19) and the majority of biomass per m^2 was between 0 and 500 g (Figure 15). A significant difference was detected in the median percent vegetative cover among lakes. Multiple comparison analyses revealed that significant differences were present between Lake Geneva (median=87.5%) and both Lake Darling (median=30%; P<0.05) and Lake Carlos (median=45%; P<0.05), but not Lake Le Homme Dieu (median=65%; P>0.05; Figure 16). Furthermore, vegetative cover was not a good indicator of zebra mussel density and was not correlated (P=0.62; Figure 17).

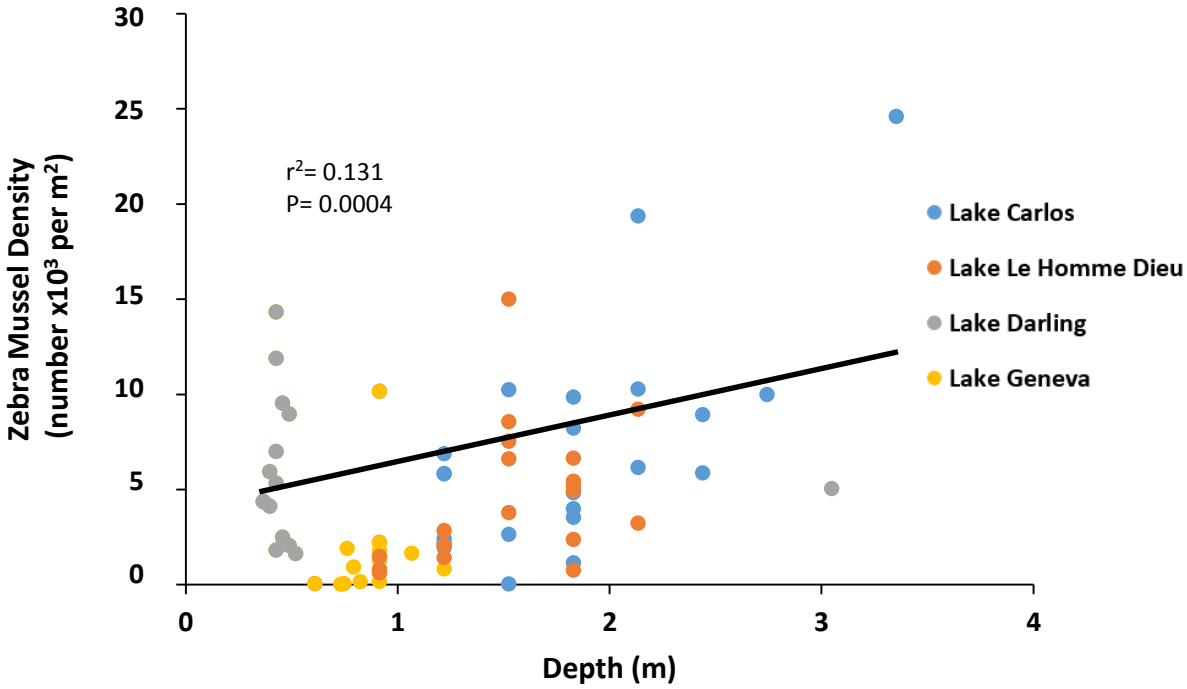

Figure 14. Zebra mussel density (Number x 10^3 per m^2) as a function of depth (m) for four west-central Minnesota lakes in the summer of 2015. Regression indicates a positive correlation between variables (P=0.0004). Blue, orange, grey, and yellow dots represent data pairings from lakes Carlos, Le Homme Dieu, Darling, and Geneva, respectively. The r^2 and P-values are denoted. N=30 for each lake.

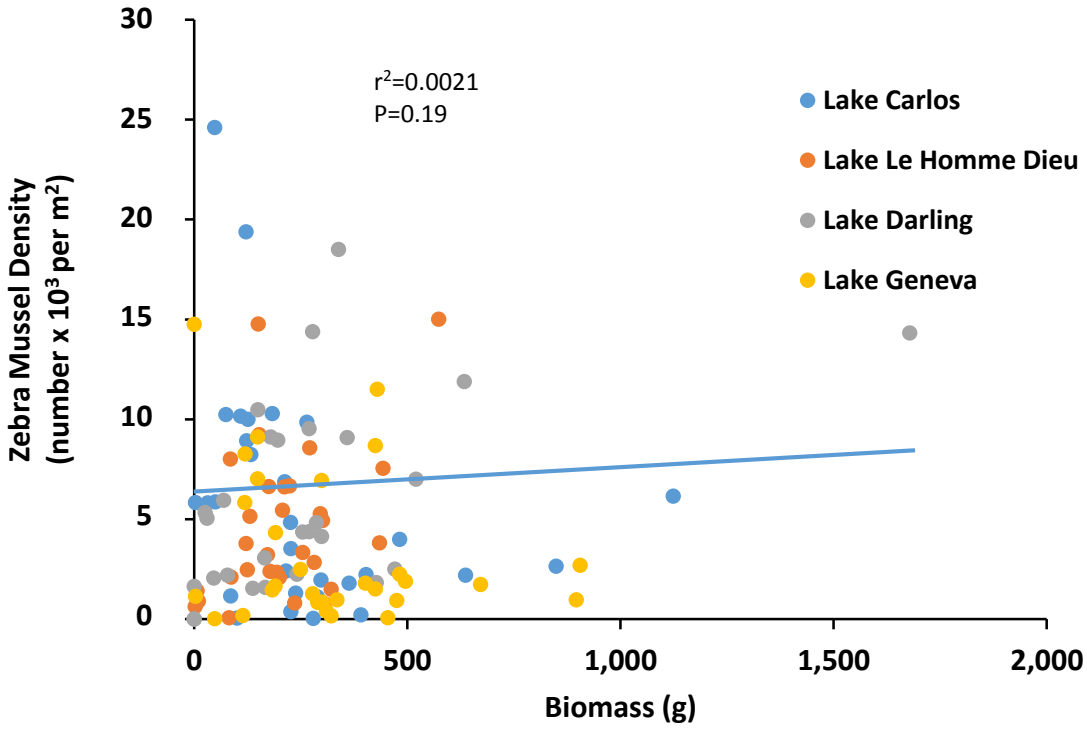

Figure 15. Zebra mussel density (number x 10^3 per m^2) as a function of organic biomass (g dry weight) for four west-central Minnesota lakes in the summer of 2015. Regression indicated no relationship between the variables (P=0.62). Blue, orange, grey, and yellow dots represent data pairings from lakes Carlos, Le Homme Dieu, Darling, and Geneva, respectively. The r^2 and P-values are denoted. N=30 for each lake.

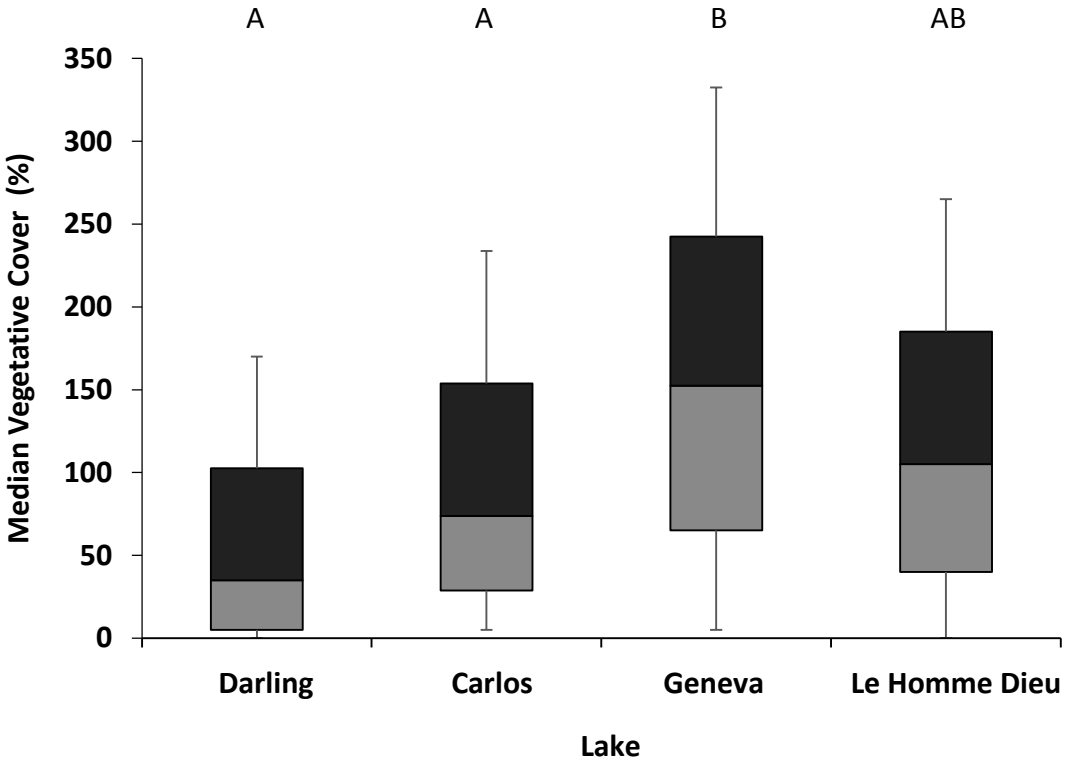

Figure 16. Macrophyte and algae (vegetative) cover (%) for four lakes in west-central Minnesota in the summer of 2015. The box plots represent the median, 25th and 75th percentiles, and the range of data (vertical bars). Significant differences are indicated by differing letters above each box plot (P<0.05). Lake Geneva significantly different than both Lake Darling and Lake Carlos but not Lake Le Homme Dieu (P=<0.05).

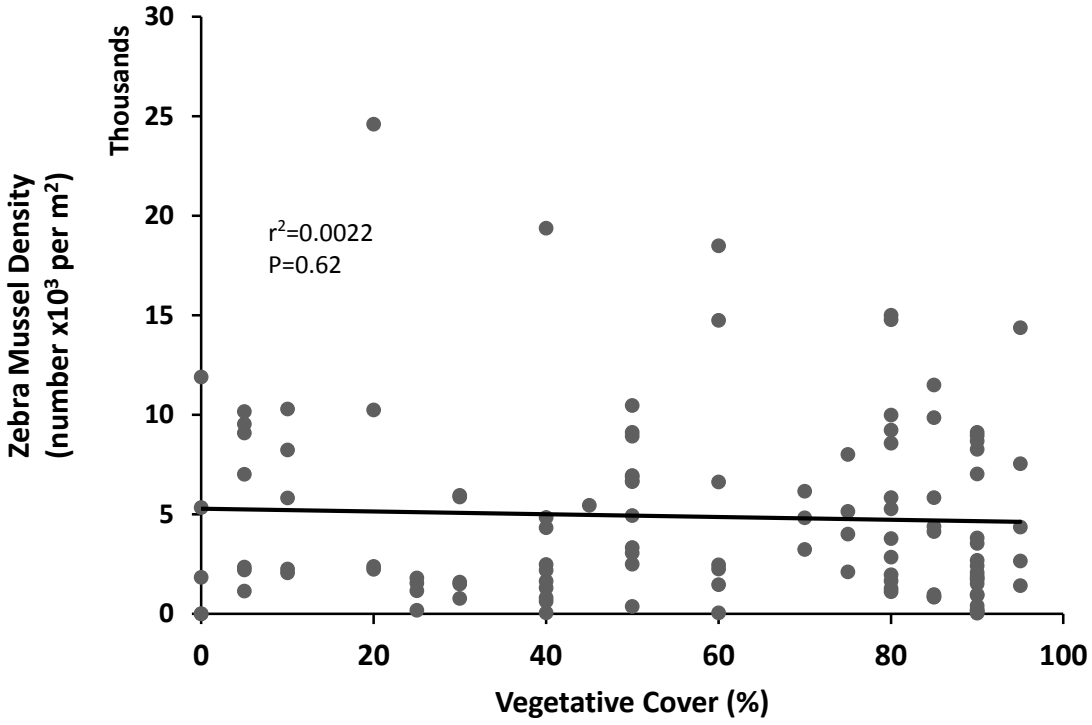

Figure 17. Zebra mussel density (number x $10^3/m^2$) in relationship to vegetative (macrophytes and algae) cover (%) for four infested lakes in west-central Minnesota in the summer of 2015. Zebra mussel densities and vegetative cover were estimated based on observations from thirty 0.25-m quadrats from each lake, thus N=120. The r^2 and P-values are denoted.

Twenty two different types of biotic substrate were found in the four lakes sampled, detritus and other (plastic and aluminum) substrates were found as well. A total of 16,334 zebra mussels were found attached to organic biomass, resulting in an overall density of 9.07/g dry weight. Of the attached mussels located, 66% were juveniles and 34% adults. Of the 22 organic substrate types, allochthonous material (*i.e.*, detritus) had the most attached zebra mussels at 896. As a side note, 394 zebra mussels were found attached to foreign debris (*e.g.,* plastic bottles and aluminum cans). Multiple comparison analyses revealed differences in median zebra mussel densities per unit of organic biomass among lakes. The median attached zebra mussels per organic biomass was significantly higher in Lake Darling (median=1.36/per g dry weight) than Lake Geneva (median= 0.00; $P<0.05$) and Lake Le Homme Dieu (median=0.00; $P<0.05$), but not Lake Carlos (median=0.109; $P>0.05$). Lake Geneva was statistically different than all three of the other lakes, but Lake Carlos and Lake Le Homme Dieu were not different from each another (Figure 18).

The LEI results indicated the potential for some electivity differences among the categories of organic biomass (Table 6). Detritus had a LEI of 0.15, suggesting that some affinity for this organic biomass type was present, but not likely being selected at a significant level over the other categories. Some electivity was also present for Fries pondweed (*Potamogeton friesii*) with an LEI value of 0.14. Fries pondweed had a total biomass of 47.43 g, with the majority of attached zebra mussels being juveniles (65%). *Najas* spp. was slightly selected with an LEI score of 0.06.

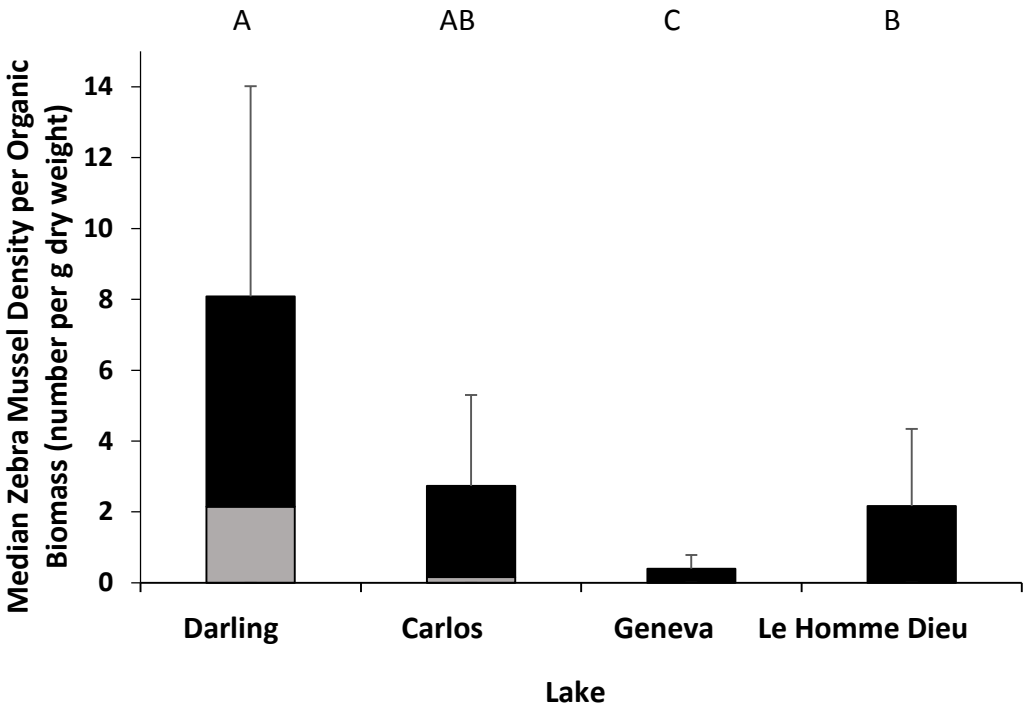

Figure 18. Zebra mussel density per unit of organic biomass (number per g dry weight) for four infested lakes in west-central Minnesota in the summer of 2015. The box plots represent the median, 25th and 75th percentiles, and the range of data (vertical bars). Zebra mussel densities and organic biomass measurements were estimated based on observations from thirty 0.25-m quadrats from each lake, thus N=120. Lakes with different letters are significantly different from each other ($P<0.05$).

Table 6. LEI index for each taxa observed. Negative values denote avoidance and positive values indicate preference.

Habitat Taxa	LEI
Filamentous algae	-0.21
Taxiphyllum spp.	0.00
Chara spp.	-0.30
Potamogeton richardsonii	0.01
Utricularia vulgaris	0.01
Ceratophyllum demersum	0.02
Detritus	0.18
Elodea canadensis	0.02
Potamogeton zosteriformes	0.03
Potamogeton friesii	0.15
Heteranthera dubia	-0.01
Potamogeton illinoensis	0.02
Najas spp.	0.07
Myriophyllum spicatum	0.03
Eleocharis acicularis	-0.00
Ruppia spp.	-0.00
Stuckenia pectinata	0.03
Potamogeton gramineus	0.00
Vallisneria americana	-0.03
Renunculus peltatus	0.00
Bidens beckii	-0.00
Potamogeton praelongus	-0.00
Nymphea odorata	NA
Other (Plastic & aluminum)	NA

Although *Chara* spp. made up a majority of the vegetative biomass in nearly all quadrats sampled, the LEI score of -0.309 suggests avoidance or inability to attach as readily. Although there appeared to be differences in zebra mussel attachment rates among the organic biomass types, no significant differences were detected when all categories were analyzed separately. However, given that filamentous algae and *Chara* spp. were not significantly different in density from each other, the two categories were combined and assessed as algaes. Additionally, differences were absent among the *Potamogeton* spp. and these categories were also combined.

Further analyses on the combined categories revealed significant differences in zebra mussel densities between algaes and *Potamogeton* spp. and between *Potamogeton* spp. and detritus (P=0.001). Similarly, zebra mussel attachment to the algaes was significantly different from the macrophytes (P<0.001).

Algaes biomass was significantly different than all taxa except water celery (*Vallisneria Americana*; P=0.001). Filamentous algaes, *Chara* spp., *Ceratophyllum demersum*, detritus, *Najas* spp., *Myriophyllum spicatum,* water celery, and *Potamogeton* spp. each made up more than 1% of the total biomass and were included in subsequent analyses (Figure 19).

A chi-square revealed the differences between observed and expected frequencies of juveniles per unit of organic biomass were not significantly different (P>0.05), but that the differences were significantly different for adults. When analyzing adult zebra mussel per g dry weight, the only lake that was shown to be significantly

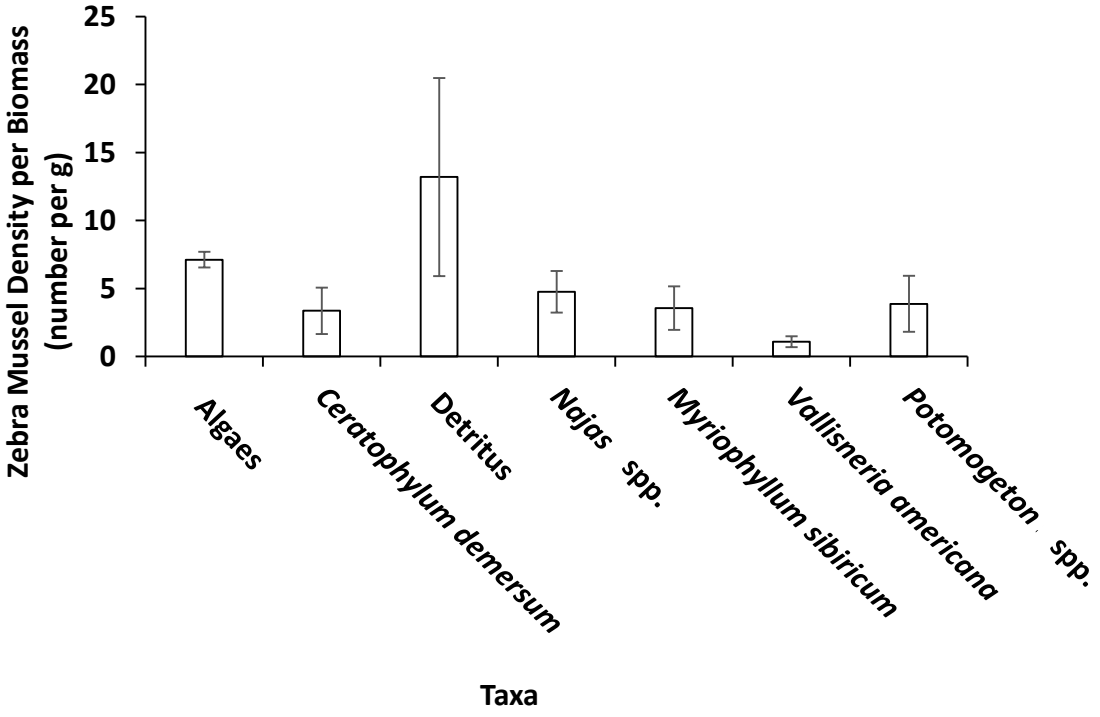

Figure 19. Mean number zebra mussels per gram of taxa category. Taxa includes vegetation, algae and detritus. Error bars indicate standard error per taxa. Each taxa represents at least 1% of the total biomass. Other taxa were recorded but not analyzed.

different from the others was Lake Geneva (median=0.00; P<0.05), with over 50% of the organic biomass samples having no adult zebra mussels (Figure 20).

When all organic biomass categories were analyzed, no differences in zebra mussel densities among the categories could be detected (P>0.05). However, when categorized into groupings making up more than 1% of the total organic biomass, adult zebra mussels were found at significantly higher densities on algaes than *Potamogeton* spp. (P<0.001), but not detritus (P>0.05). Furthermore, detritus and *Potamogeton* spp. were not significantly different (P>0.05). The adults attached to algaes and detritus were also significantly different from macrophytes (P<0.001), but not each other (P>0.05).

Conversely, there were significant differences in the number of juveniles attached to organic biomass among lakes. Juvenile zebra mussels attached less often in Lake Geneva (median=0.00) than Lake Darling (median=1.89; P<0.05) and Lake Carlos (median=0.193; P<0.05); however, lakes Darling and Carlos were not different (P>0.05). Lake Le Homme Dieu was not significantly different from any of the other lakes (median=0.00; P>=0.05; Figure 20).

There was not a significant difference among juveniles per organic biomass on all taxon separately, but when combined, there was a significant difference among algaes, detritus, and *Potamogeton* spp. categories (P<0.001). Furthermore, the density of juveniles attached to algaes was significantly higher than all macrophytes and detritus (P<0.001), but macrophytes and detritus were not significantly different (P>0.05).

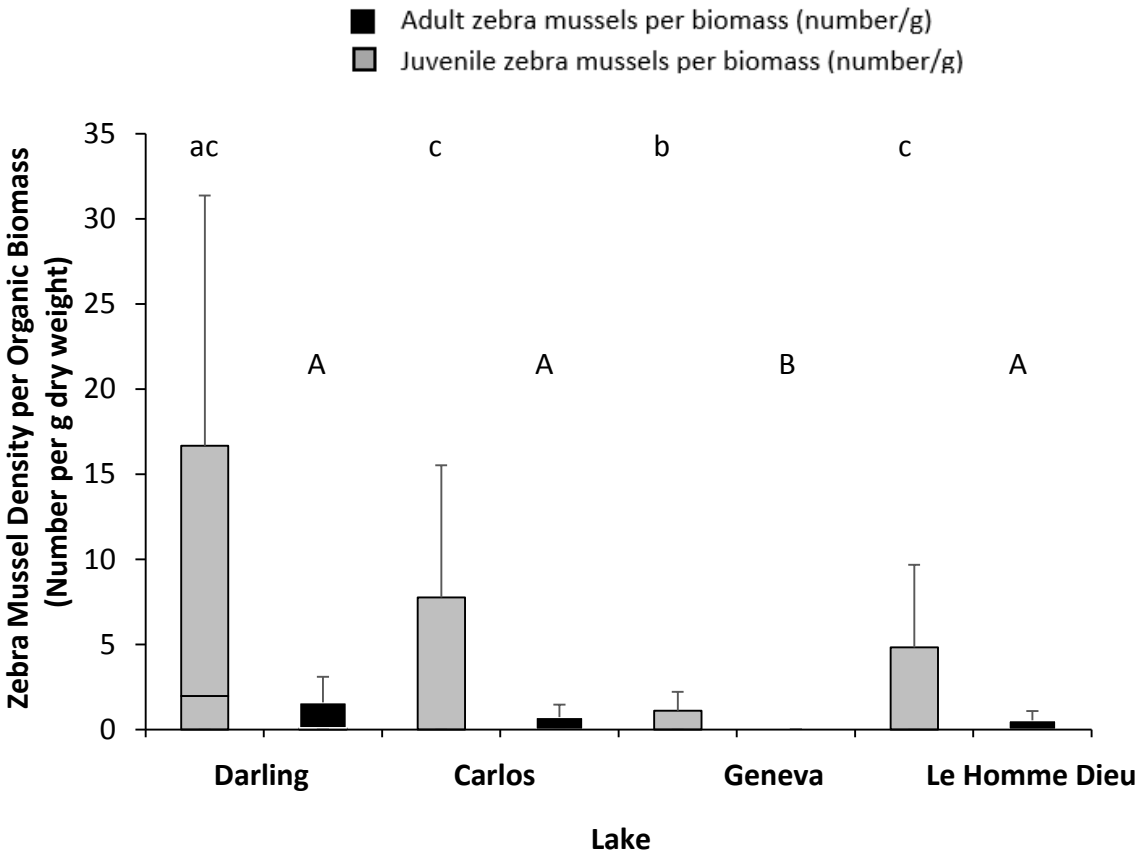

Figure 20. Adult (≥9 mm) and juvenile (≤8 mm) zebra mussel density per unit of organic biomass (#/g) for four infested lakes in west-central Minnesota in the summer of 2015. Grey bars indicate juveniles per biomass and black bars indicate adults per biomass. The box plots represent the median, 25th and 75th percentiles, and the range of data (vertical bars). Zebra mussel densities and organic biomass measurements were estimated based on observations from thirty 0.25-m quadrats from each lake, thus N=120. Capital letters indicate significance among adults and lower case letters indicate significance among juveniles.

The primary life stage found on organic biomass was juveniles, making up 68% of the zebra mussels attached. Additionally, when t-tests were run between juvenile and adult zebra mussels per different organic biomass categories, differences were present, with greater numbers of attached juveniles ($P<0.001$). Likewise, significant differences between juvenile and adult zebra mussel densities were also present on the combined categories of algaes, *Potamogeton* spp., and macrophytes ($P<0.05$). Detritus had the most juvenile attachment per organic biomass, while *Myriophyllum spicatum* had the second most juveniles per biomass, but the lowest number of adults (Table 7).

Objective 3

Out of the four lakes sampled, 3 contained native mussel whole shells and all the lakes sampled contained native mussel shell pieces. Whole native mussel shells were not found in Lake Geneva. Two native mussel species were found Fatmucket (*Lampsilis siloquoidea)* and giant floater (*Pyganodon grandis*), both from family Unionidae. Lake Carlos consisted of 2 dead *L. siloquoidea* whole shells and 5 out of the 30 samples from Lake Carlos contained native mussel fragments and/or whole shells. Sixty percent of native mussel shells in Lake Carlos contained attached zebra mussels. No *P. grandis* specimens were found in Lake Carlos. Lake Le Homme Dieu consisted of 2 dead *L. siloquoidea* shell fragments, both of which had attached zebra mussels. Two out of 30samples in Lake Le Homme Dieu contained native mussel shell fragments. One live *L. siloquoidea* was found in Lake Darling, that when extrapolated out, suggests an estimated population of 89,552 within the 50-m buffer sampled. A total of 15 dead *L.*

Table 7. Number, biomass, Number per biomass, expected juveniles per biomass, and expected adults per biomass per taxa category. Each taxa represents at least 1% of the total biomass. Other taxa were recorded but not analyzed. Expected juveniles and expected adults were determined by using proportions of the observed zebra mussels.

Taxa	Number per g	number	g	SE	Expected Juv per g	Expected Adult per g
Filamentous Algae	4.05	344.86	133.27	0.84	10.16	1.96
Chara spp.	3.07	300.49	173.96	0.79	8.43	1.38
Algae	7.12	645.34	307.23	0.58	18.59	3.35
Ceratophylum demersum	3.37	55.88	25.17	1.70	12.51	0.78
Detritus	13.20	100.85	52.91	7.28	25.65	7.71
Najas spp.	4.77	40.56	20.78	1.53	11.01	2.25
Myriophyllum sibiricum	3.57	33.06	26.50	1.60	13.45	0.41
Vallisneria americana	1.09	35.29	44.20	0.40	2.04	0.58
Potamogeton spp.	3.88	14.43	9.59	2.05	7.24	0.75

siloquoidea whole shells were found, of which 65% were serving as zebra mussel substrate. Four *P. grandis* specimens were found in Lake Darling, of which only 1 was free from zebra mussels. Fifty percent of Lake Darling samples contained whole native mussel shells and/or native mussel shell fragments (Table 8).

Overall, 18% of the quadrats contained native mussel whole shells or fragments. Mean native mussel length was 64.76 mm and was serving as the substrate for an average of 148 zebra mussels. Zebra mussel size was not dependent on length of native mussels (P= 0.59; Figure 21), with a maximum length of 19.48 and a minimum of 8.71. Mean size between zebra mussels attached to native mussels and attached to other substrate (*i.e.,* rocks, vegetation, detritus) was not statistically different (P=0.65). Chinese mystery snails (*Cipangopaludina chinensas*), another invasive mollusk belonging to the family Viviparidae was found in 11 of the 30 quadrats in Lake Le Homme Dieu. Of the Chinese mystery snails located, 77% had zebra mussels attached. The mean zebra mussel length attached to this species was 10.95 mm; however, the number and length of zebra mussels were not significantly different between native mussel shells and *C. chinensas.*

Table 8. Summary table of the native mussels found in four west-central Minnesota study lakes in the summer of 2015. The species identified and total number collected (dead + living specimens), and notes about what was found are included.

Lake	Native mussel species (# collected)	Notes
Carlos	*Lampsilis siloquoidea* (2); *Pyganodon grandis* (0)	5 of 30 quadrats had native mussels 0 live/2 dead
Darling	*Lampsilis siloquoidea* (16); *Pyganodon grandis* (4)	15 of 30 quadrats had native mussels 1 live/18 dead
Geneva	None	0 of 30 quadrats had native mussels
Le Homme Dieu	*Lampsilis siloquoidea* (2); *Pyganodon grandis* (0)	2 of 30 quadrats had native mussels 0 live/2 dead

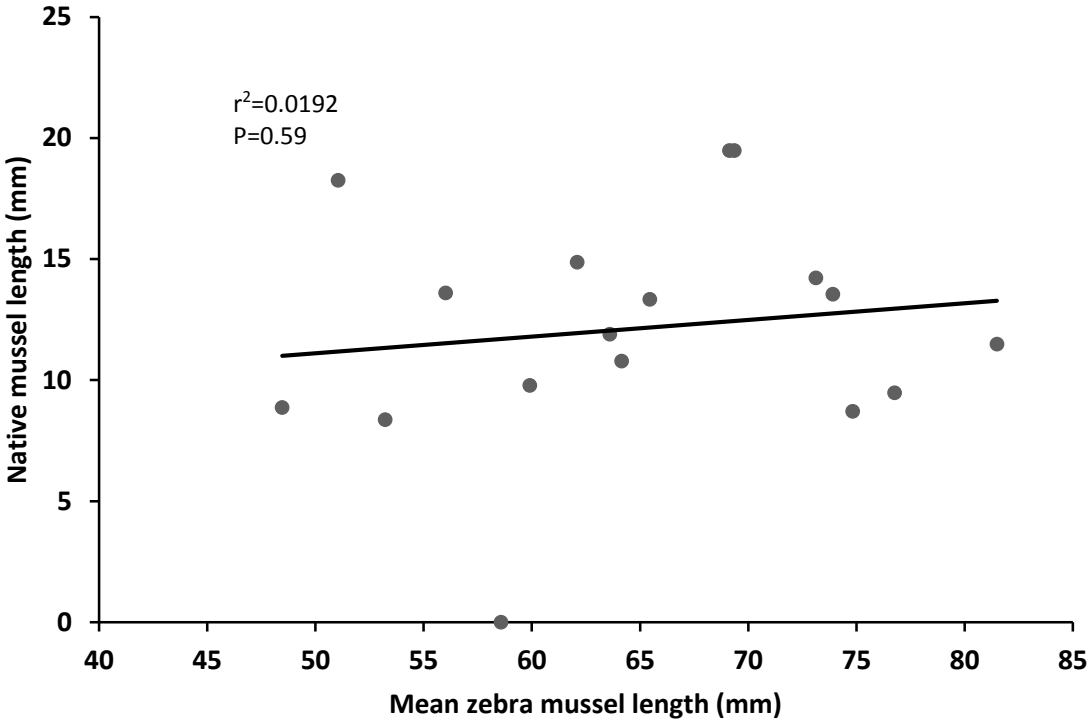

Figure 21. Mean native mussel length per mean zebra mussel length regression results for four west-central Minnesota lakes for data collected in the summer of 2015.

Individual Lake Analyses

As was reported above for the four study lakes collectively, each lake was also evaluated individually. It should be noted that in Lake Darling, two outliers were detected (Figure 22) and removed from subsequent analyses, however, there was still no correlation (P=0.98) between zebra mussel density and phi (ϕ) values (Figure 23). In Lake Darling, no significant correlation (P=0.177) was present between zebra mussel density and organic biomass when outliers were excluded (Figures 24 and 25). When outliers were included, there was a significant correlation (P= 0.024) between zebra mussel density and biomass (Figure 26); however, the outliers expressed considerable weight in the results. The results of the individual lake assessments are summarized in Table 9 and Figures 27-39.

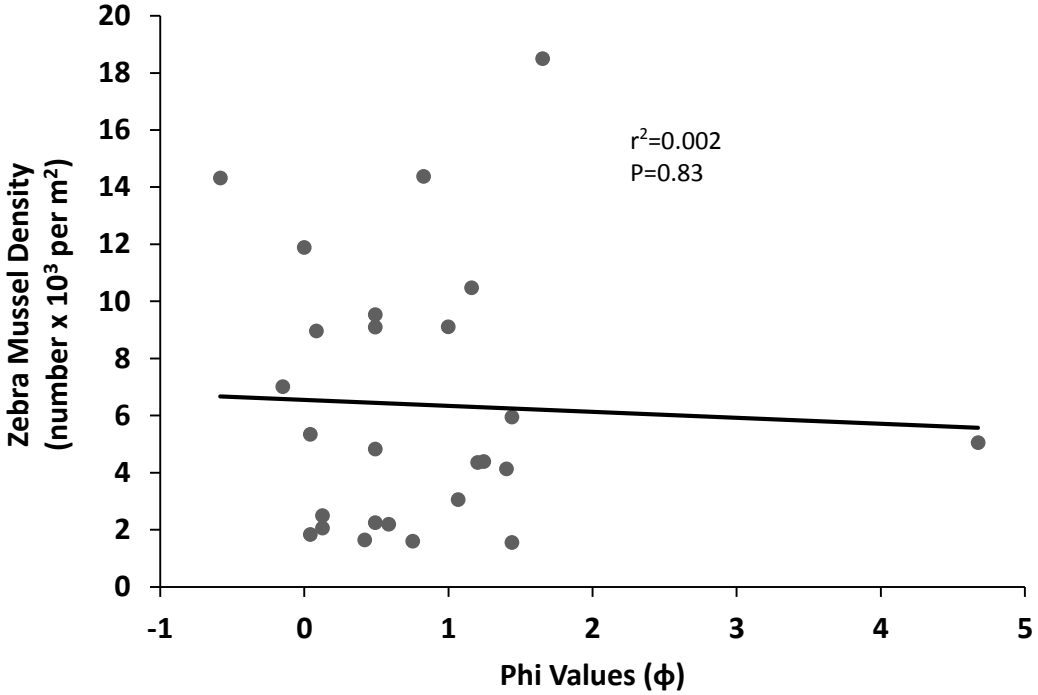

Figure 22. Zebra mussel density (Number x 10^3 per m^2) as a function of phi value (ϕ) in Lake Darling, Minnesota from the summer of 2015. Negative phi values indicate larger substrates such as boulders and gravel. Positive phi values indicate smaller substrates such as, sand, silts, and clays. The r^2 and P-values are denoted (N=28).

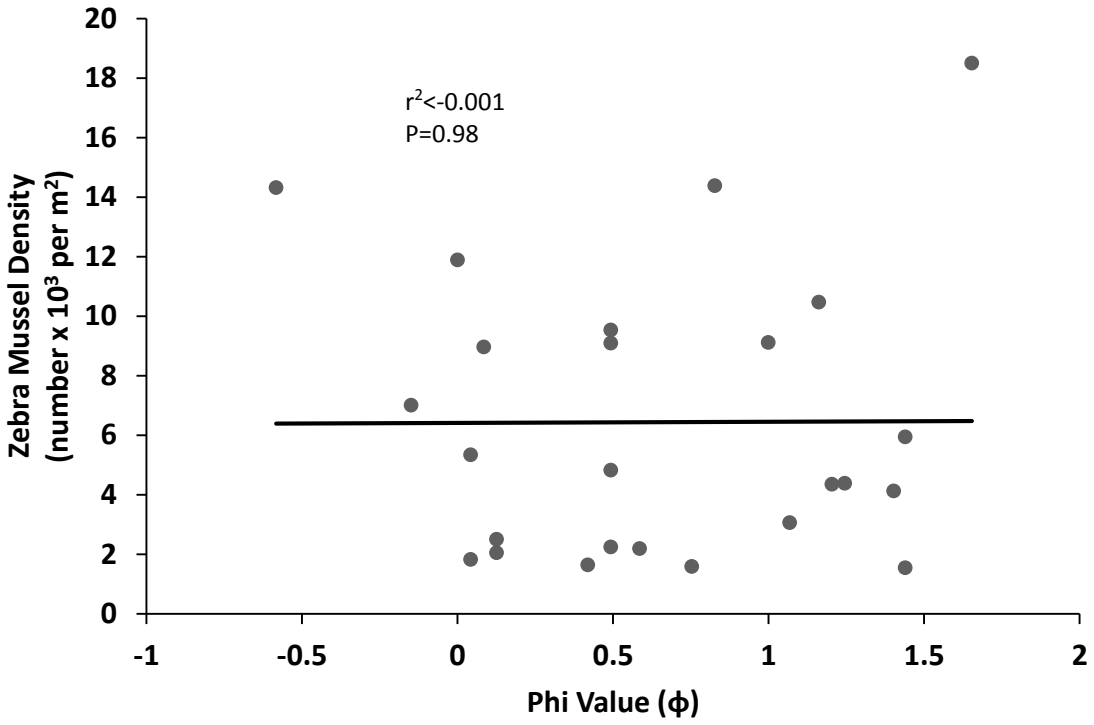

Figure 23. Zebra mussel density (Number x 10^3 per m^2) as a function of phi value (ϕ) in Lake Darling, Minnesota from the summer of 2015 and after outliers removed. Negative phi values indicate larger substrates such as boulders and gravel. Positive phi values indicate smaller substrates such as, sand, silts, and clays. The r^2 and P-values are denoted (N=26).

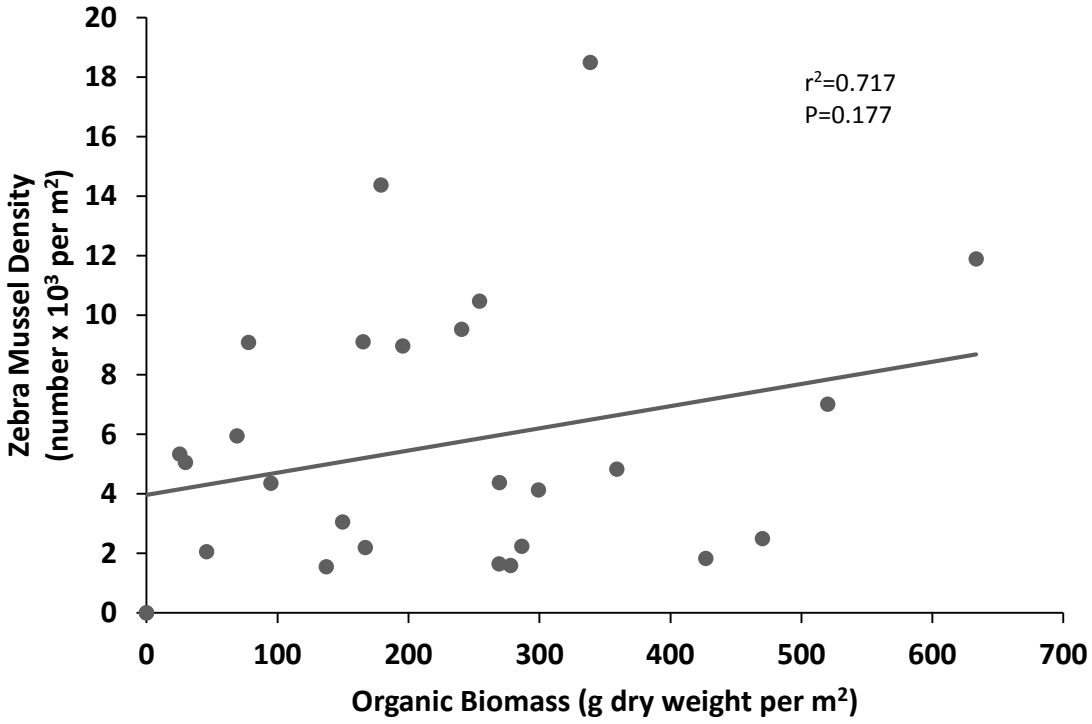

Figure 24. Zebra mussel density (number x 10^3 per m^2) as a function of organic biomass (g dry weight) for Lake Darling, Minnesota in the summer of 2015 after outliers were removed. The r^2 and P-values are denoted (N=26).

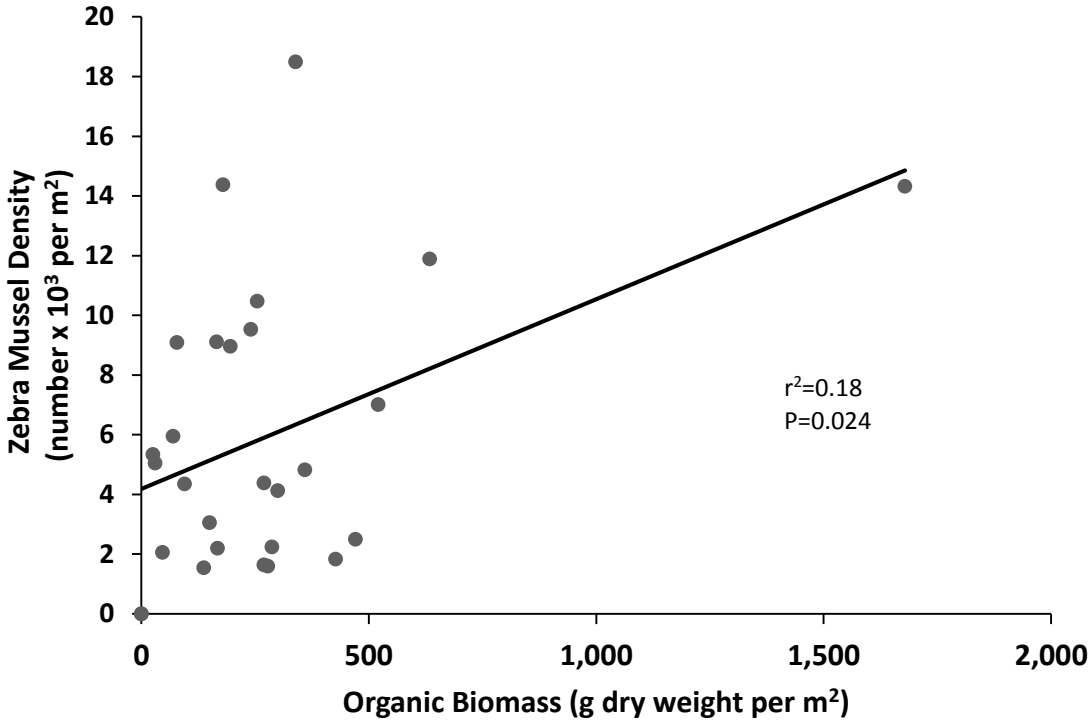

Figure 25. Zebra mussel density (Number x 10^3 per m^2) as a function of organic biomass (g dry weight) for Lake Darling, Minnesota in the summer of 2015. The r^2 and P-values are denoted (N=28).

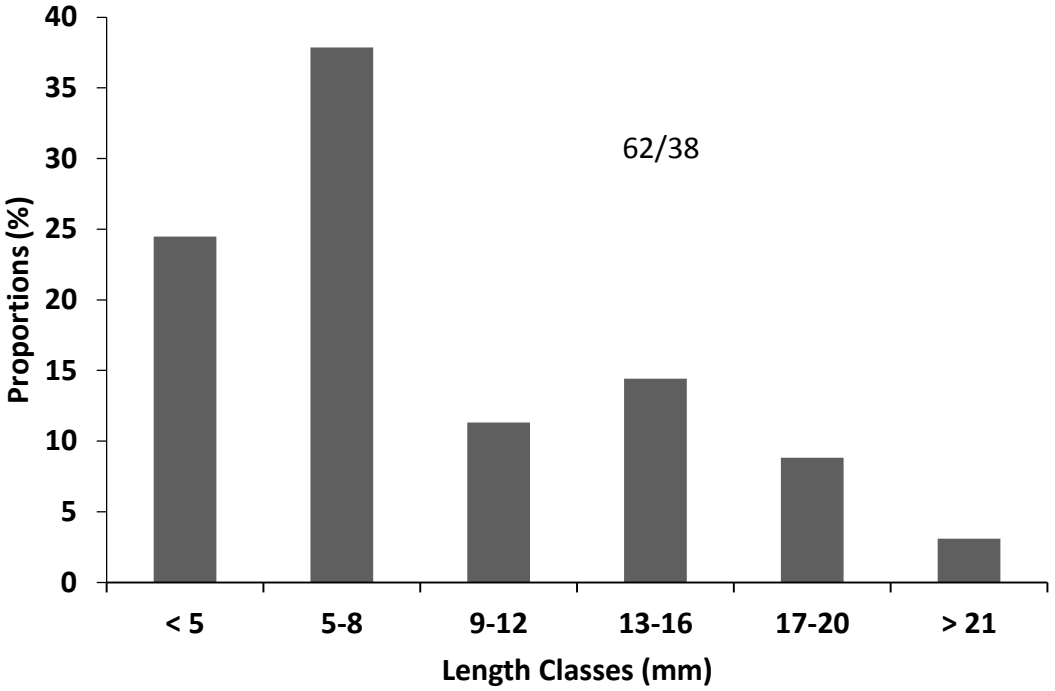

Figure 26. Proportion of zebra mussels per length class (mm) in Lake Darling, Minnesota from the summer of 2015. Juvenile zebra mussels were (≤8mm) and adults (≥9 mm). Ratio of juveniles to adults was 62/38.

Table 9. Summary statistics for various parameters observed and evaluated at four study lakes in west-central Minnesota in the summer of 2015. The table includes individual lake data for zebra mussel (ZM) population measures, geologic substrates, vegetative cover, and organic biomass. Also denoted below are the results of regression analyses assessing ZM densities as functions of phi (ϕ) value, vegetative cover (%), and organic biomass density ($g \cdot m^{-2}$) dry weight.

	Study Lake Name			
Parameter and Summary Statistics	Carlos	Darling*	Geneva	LHD**
# Quadrats (N)	30	28	30	30
Zebra Mussel Population				
# ZMs Collected	47,098	45,187	27,178	37,823
Overall Density (#/m^2)	6,280	6,455	3,623	5,043
Density (#x10^3/m^2) Range	0.04 to 24.60	1.55 to 18.50	0.03 to 11.50	0.07 to 15.00
Life Status (% alive/% dead)	72/28	71/29	36/64	65/35
Lengths (%≤8 mm/%>8 mm)	57/43	62/38	69/31	54/44
Juvenile:Adult Ratio	1.3:1	1.6:1	2.2:1	1.2:1
Geologic Substrates				
Mean Phi (ϕ) Value	-0.19	0.79	0.59	0.60
Phi (ϕ) Value Range	-5.47 to 1.19	-0.58 to 4.68	0.00 to 2.54	-0.72to 3.93
% Phi Values <0 (coarse substrates)	16	7	0	13
% Phi Values >0 (fine substrates)	84	93	100	87
Phi Value-ZM Correlation	r^2=0.033 P=0.16	r^2=0.002 P=0.83	r^2=0.001 P=0.48	r^2=0.043 P=0.27
Vegetative Cover				
Mean Cover (%)	38	50	76	59
Cover (%) Range	5 to 95	0 to 95	5 to 90	0 to 95
Vegetative Cover-ZM Correlation	r^2=0.101 P=0.87	r^2=0.091 P=0.13	r^2=0.002 P=0.95	r^2=0.228 P<0.01
Organic Biomass				
Mean biomass Density (g/m^2)	263.67	255.38	323.65	207.66
Biomass Density (g/m^2) Range	0 to 633	0 to 1,628	0 to 905	2 to 573
Mean ZM Density (#ZM/$g \cdot m^{-2}$)	6.91	9.65	0.67	5.96
Organic Biomass-ZM Correlation	r^2=0.072 P=0.18	r^2=0.180 P=0.024	r^2=0.066 P=0.85	r^2=0.164 P=0.03

*Data include two identified outliers, but N was reduced by 2 as the result of a lab processing error.
**Le Homme Dieu

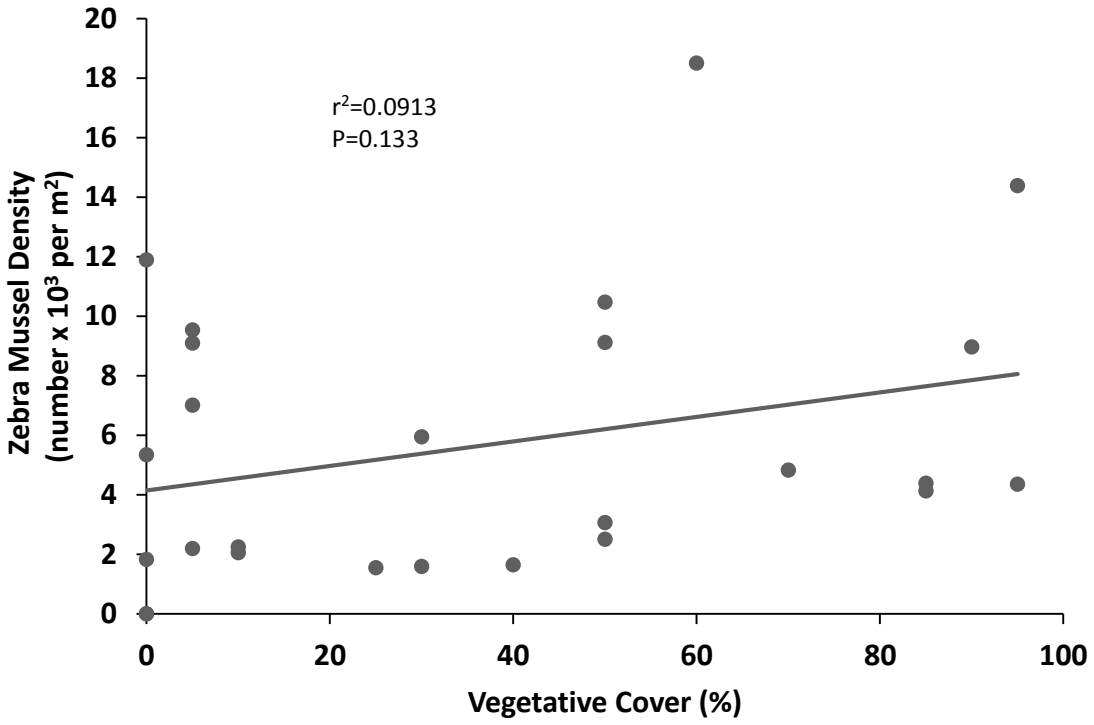

Figure 27. Zebra mussel density (number x 10^3 per m^2) in relationship to vegetative (macrophytes and algae) cover (%) for Lake Darling, Minnesota from the summer of 2015. Zebra mussel densities and vegetative cover were estimated based on observations from thirty 0.25-m quadrats. The r^2 and P-values are denoted (N=30).

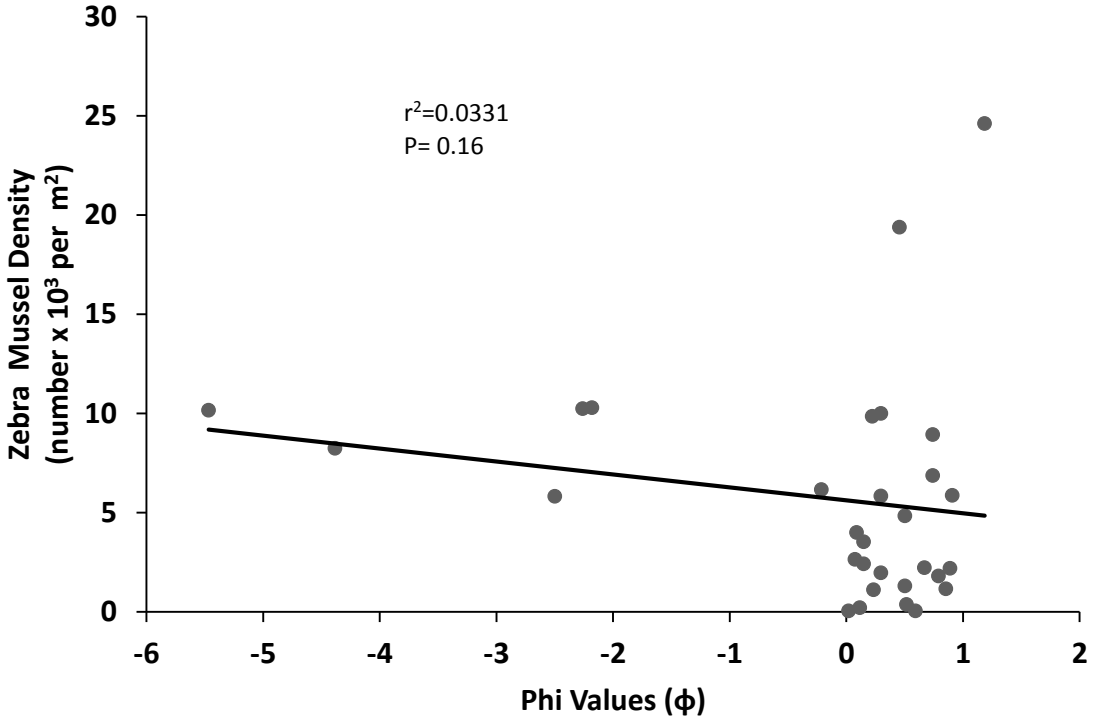

Figure 28. Zebra mussel density (Number x 10^3 per m^2) as a function of phi value (ϕ) in Lake Carlos, Minnesota from the summer of 2015. Negative phi values indicate larger substrates such as boulders and gravel. Positive phi values indicate smaller substrates such as, sand, silts, and clays. The r^2 and P-values are denoted (N=30).

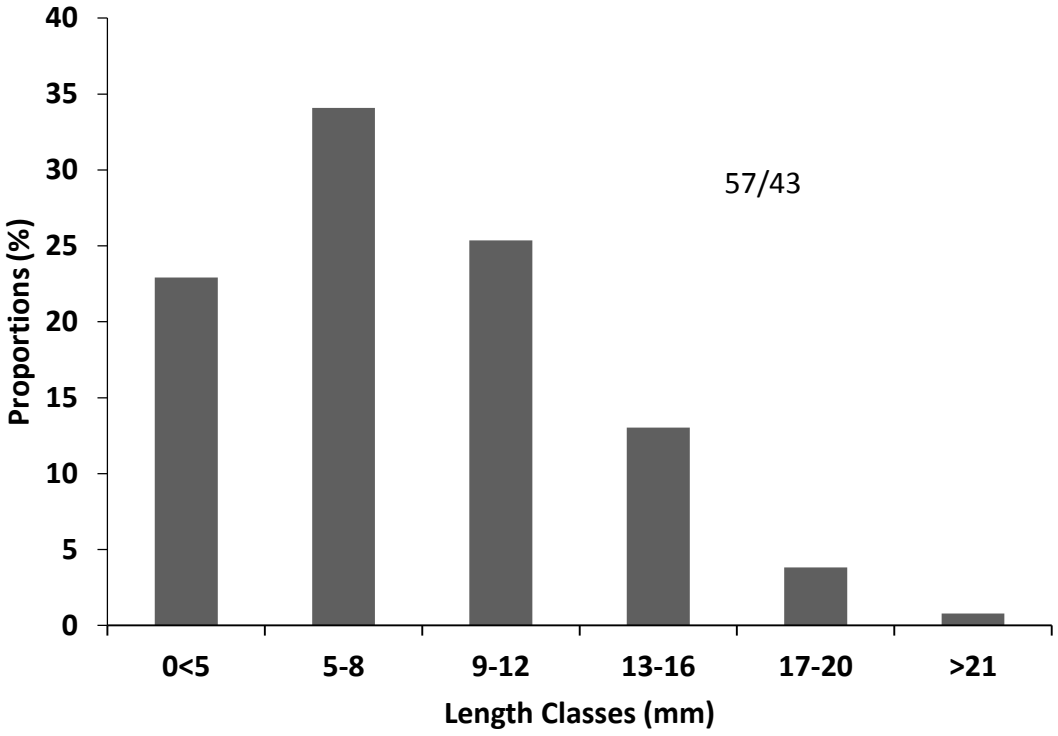

Figure 29. Proportions of zebra mussels per length class (mm) in Lake Carlos, Minnesota from the summer of 2015. Juvenile zebra mussels were (≤8 mm) and adults (≥9 mm). Ratio of juveniles to adults was 57/43.

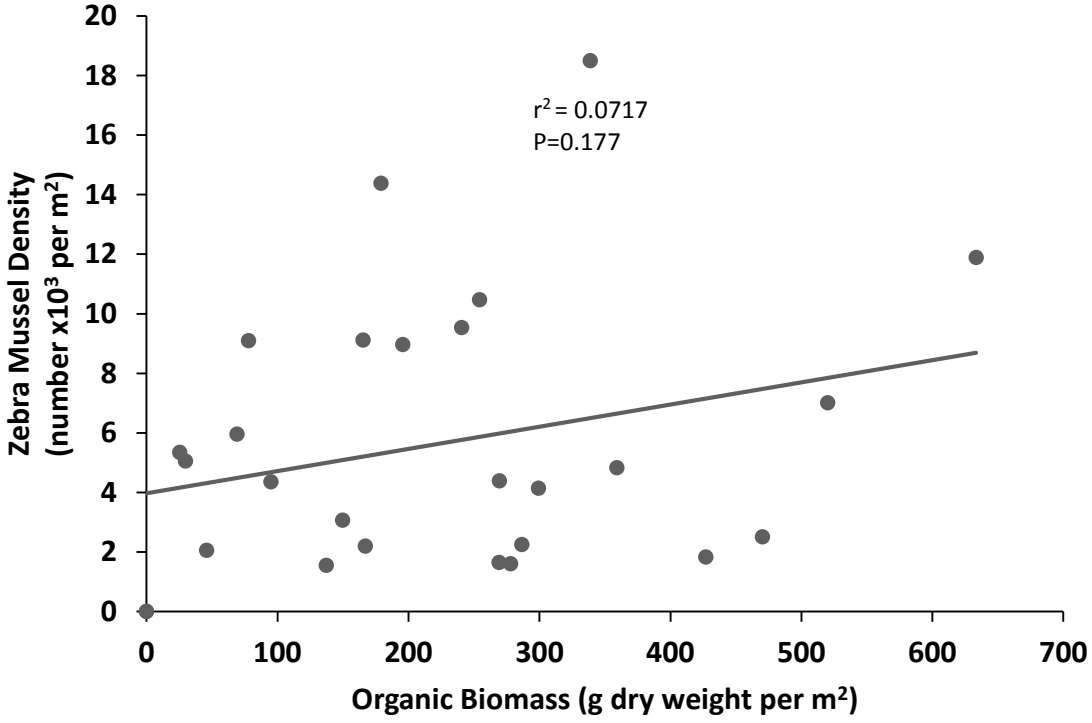

Figure 30. Zebra mussel density (number x $10^3/m^2$) as a function of organic biomass (g dry weight) for Lake Carlos, Minnesota in the summer of 2015. The r^2 and P-values are denoted (N=30).

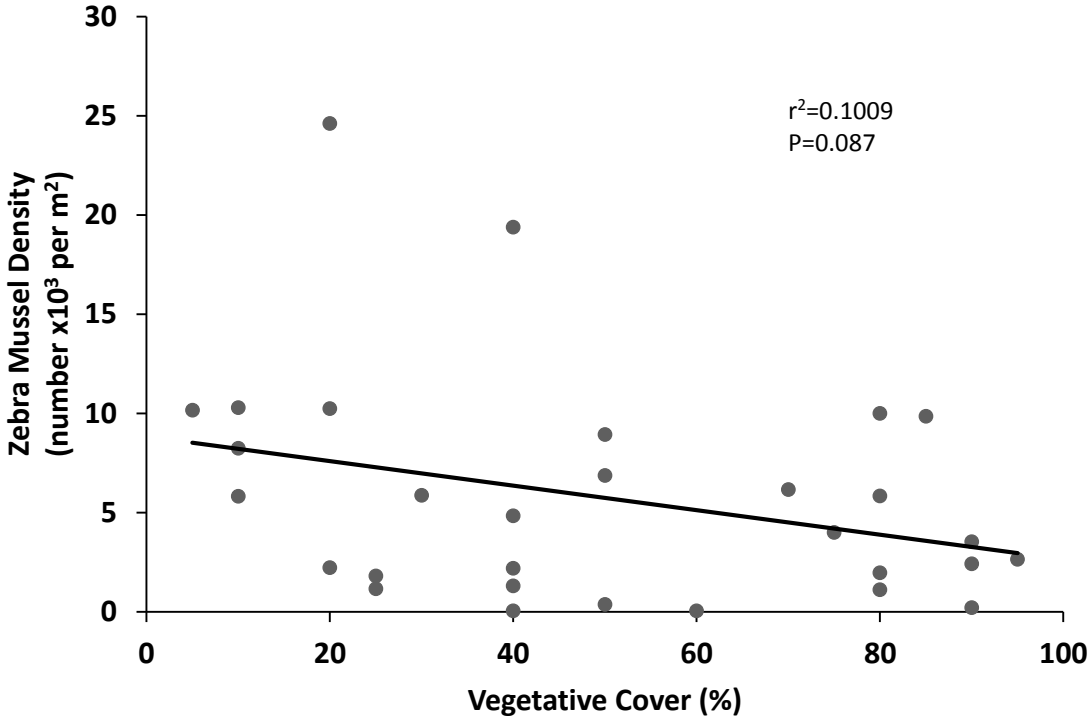

Figure 31. Zebra mussel density (number x 10^3 per m^2) in relationship to vegetative (macrophytes and algae) cover (%) for Lake Carlos, Minnesota from the summer of 2015. Zebra mussel densities and vegetative cover were estimated based on observations from thirty 0.25-m quadrats.

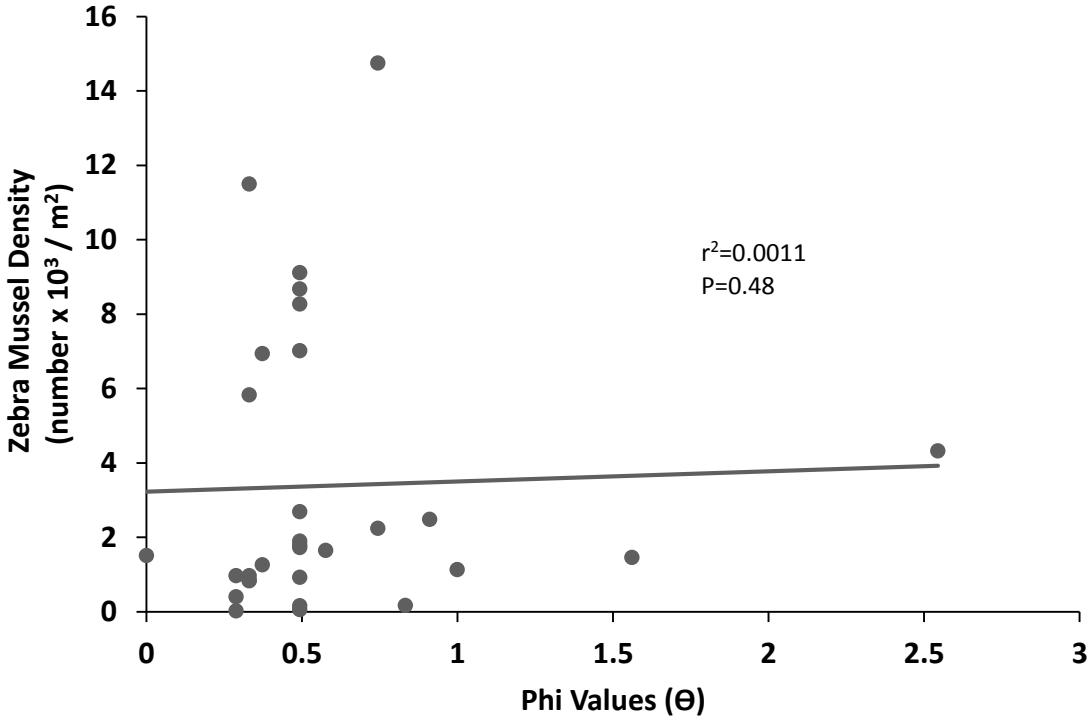

Figure 32. Zebra mussel density (Number x 10^3 per m^2) as a function of phi value (ϕ) in Lake Geneva, Minnesota from the summer of 2015. Negative phi values indicate larger substrates such as boulders and gravel. Positive phi values indicate smaller substrates such as, sand, silts, and clays. The r^2 and P-values are denoted (N=30).

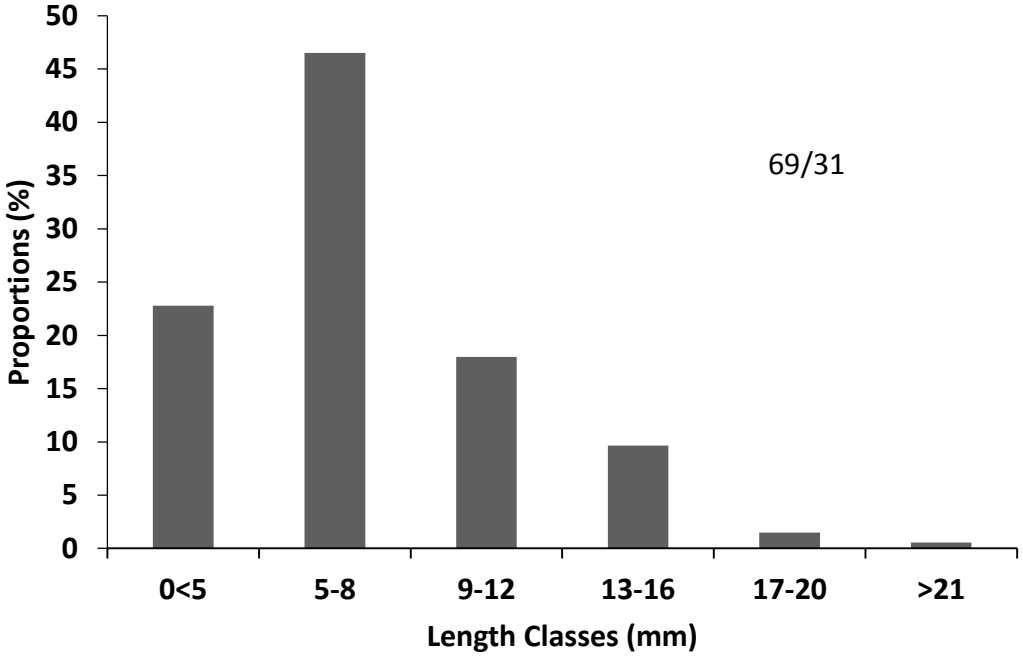

Figure 33. Proportions of zebra mussels per length class (mm) in Lake Geneva, Minnesota from the summer of 2015. Juvenile zebra mussels were (≤8mm) and adults (≥9mm). Ratio of juveniles to adults was 69/31.

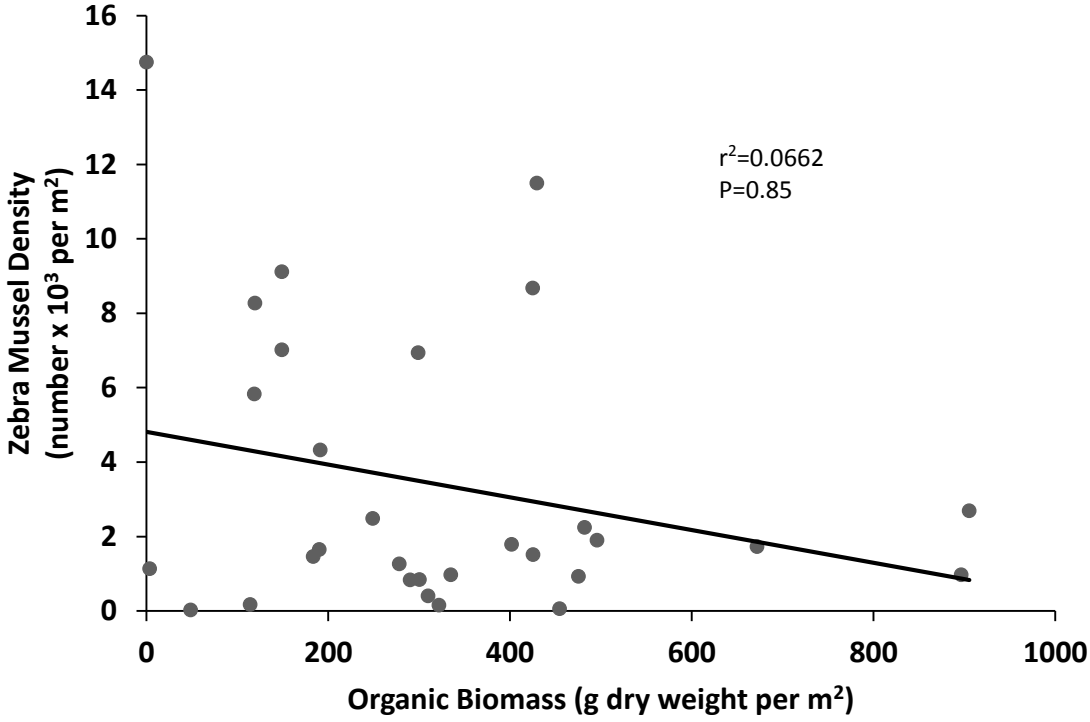

Figure 34. Zebra mussel density (Number x $10^3/m^2$) as a function of organic biomass (g dry weight) for Lake Geneva, Minnesota in the summer of 2015. The r^2 and P-values are denoted (N=30).

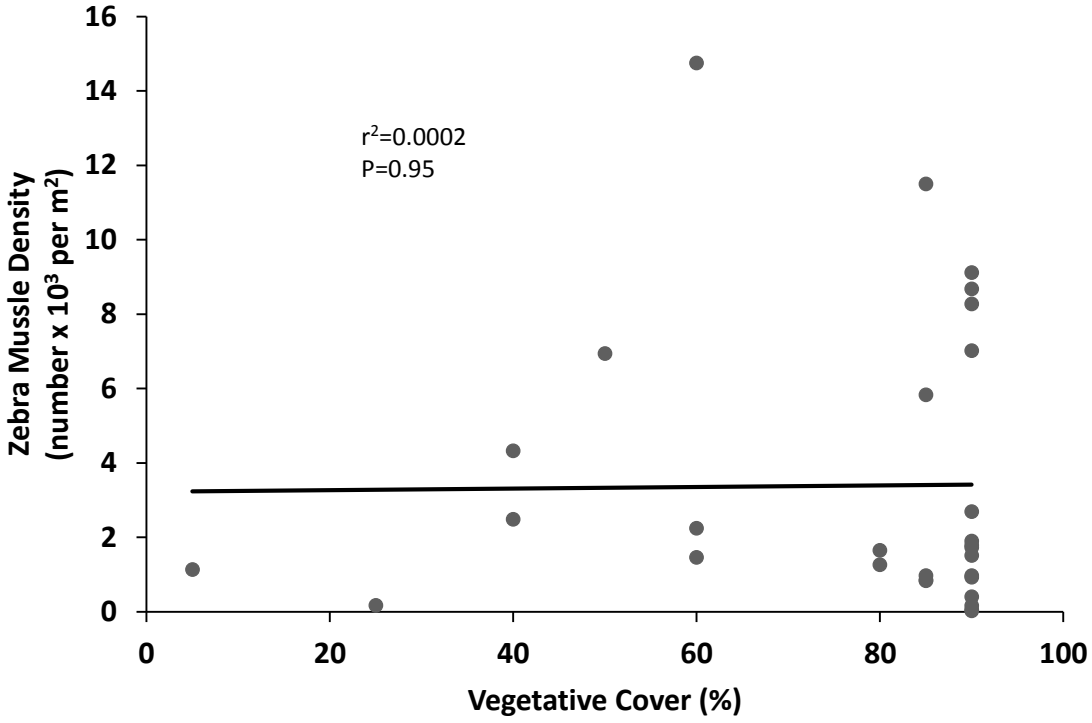

Figure 35. Zebra mussel density (number x 10^3 per m^2) in relationship to vegetative (macrophytes and algae) cover (%) for Lake Geneva, Minnesota from the summer of 2015. Zebra mussel densities and vegetative cover were estimated based on observations from thirty 0.25-m quadrats.

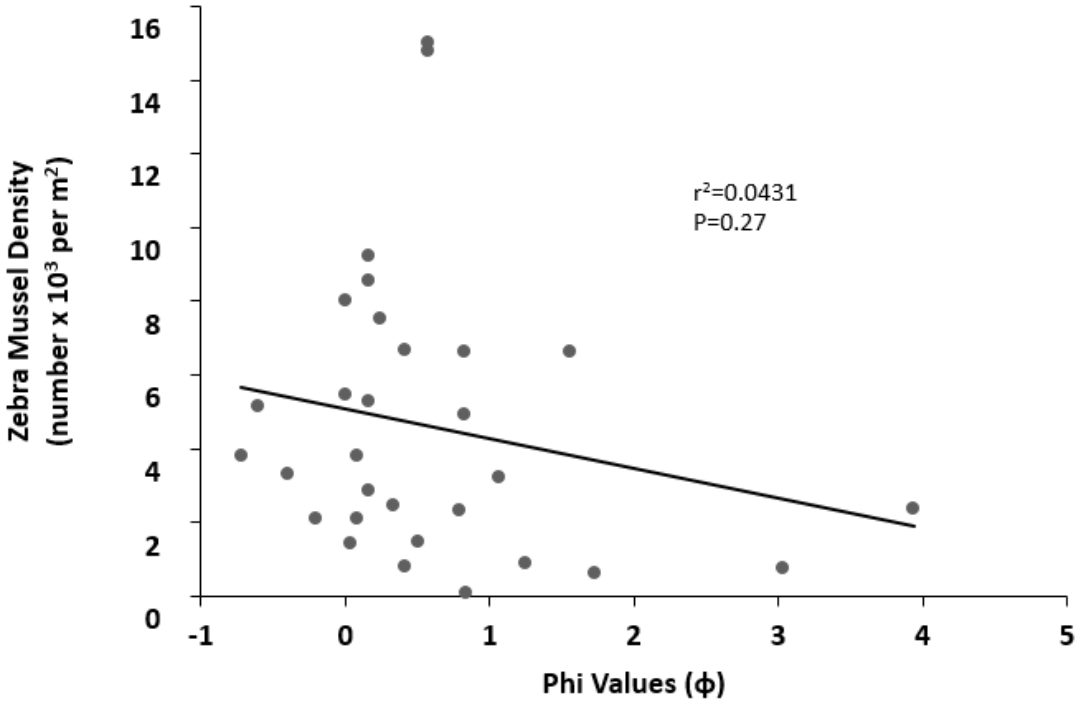

Figure 36. Zebra mussel density (number x 10^3 per m^2) as a function of phi value (ϕ) in Lake Le Homme Dieu, Minnesota from the summer of 2015. Negative phi values indicate larger substrates such as boulders and gravel. Positive phi values indicate smaller substrates such as, sand, silts, and clays. The r^2 and P-values are denoted (N=30).

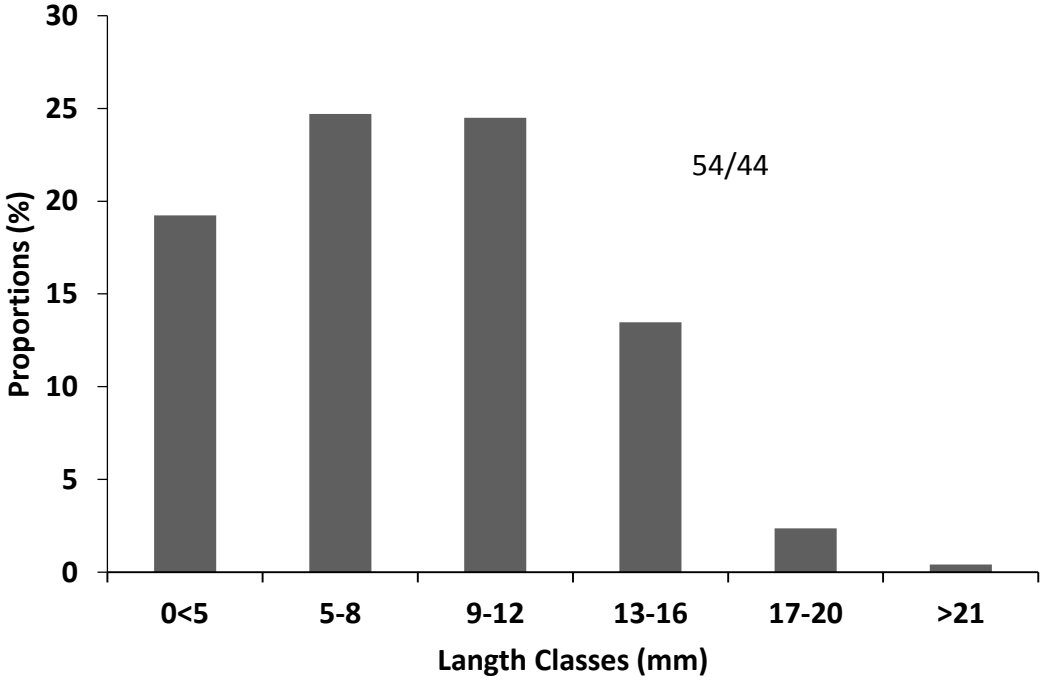

Figure 37. Proportions of zebra mussels per length class (mm) in Lake Le Homme Dieu, Minnesota from the summer of 2015. Juvenile zebra mussels were (≤8 mm) and adults (≥9 mm). Ratio of juveniles to adults was 54/44.

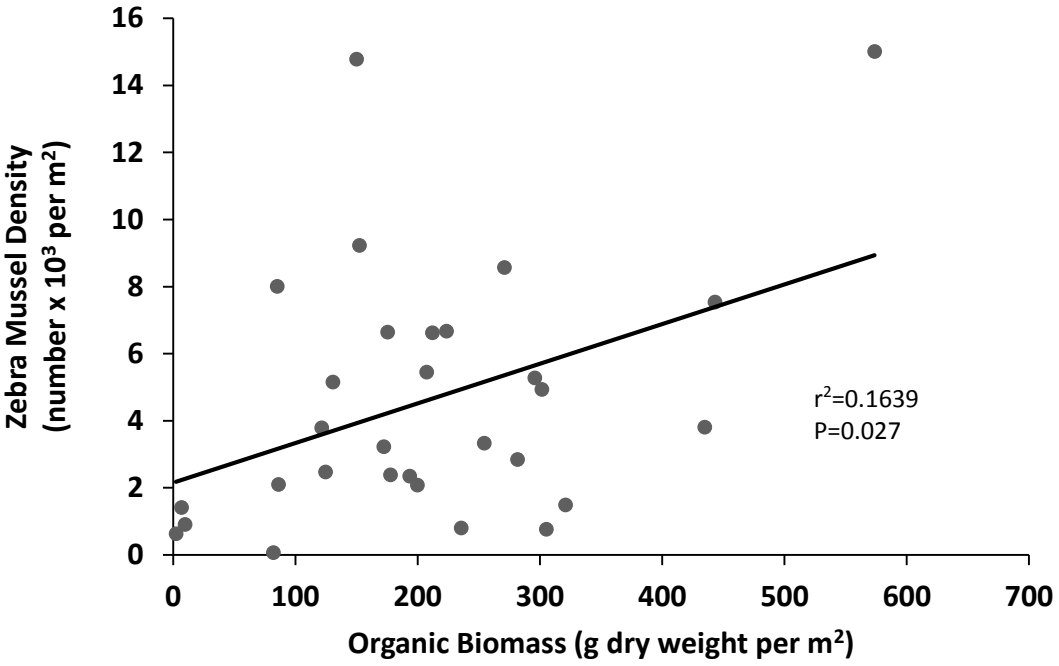

Figure 38. Zebra mussel density (number x 10^3 per m^2) as a function of organic biomass (g dry weight) for Lake Le Homme Dieu, Minnesota in the summer of 2015. The r^2 and P-values are denoted (N=30).

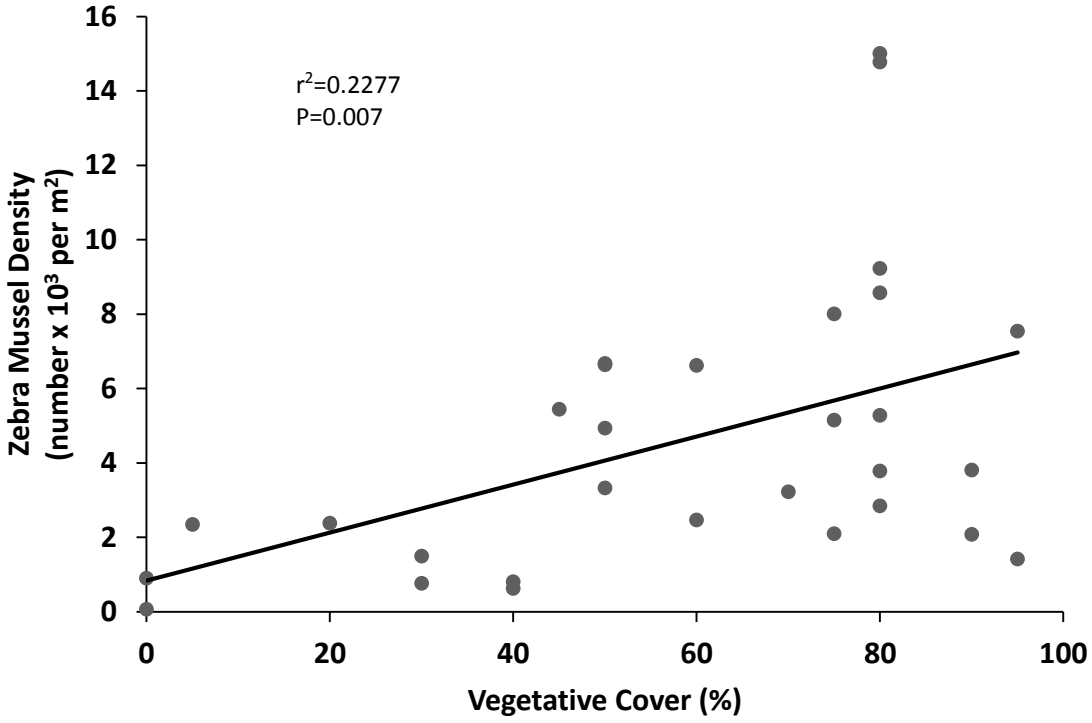

Figure 39. Zebra mussel density (number x 10^3 per m^2) in relationship to vegetative (macrophytes and algae) cover (%) for Lake Le Homme Dieu, Minnesota from the summer of 2015. Zebra mussel densities and vegetative cover were estimated based on observations from thirty 0.25-m quadrats.

Discussion and Future Research

Zebra Mussel Density as a Function of Substrate Size and Depth (Objective 1)

Nadaffi et al. (2010) noted that zebra mussels attach to a multitude of substrates and their densities vary along depth gradients. The literature also suggests that zebra mussel density is often correlated with the overall health of the zebra mussel population in a given water body. Therefore the densities found in my study lakes should be compared to other area lakes that share similar lake characteristics to better assess the potential to make predictions regarding zebra mussel densities.

Although past research has noted significant differences in zebra mussel density due to substrate size (Mellina and Rasmussen, 1994; Berkman et al. 2000), this was not the case in the present study. Furthermore, a minimal gradient of phi values was seen among lakes. To explain the lack of a relationship between zebra mussel density and the underlying geologic substrate in these lakes, I must address the geomorphology in the region.

The study lakes' headwaters are located north of Alexandria, Minnesota and are all connected via the Long Prairie River that meanders through central Minnesota. All four lakes were also carved by the Laurentide Ice Sheet (MN DNR, 2015). Therefore, the connectivity and geologic history shared by these lakes likely explains the lack of substantial variation in substrates. With minimal variation in substrate, assessing zebra mussel density as a function of substrate size was did have enough range to be overly useful. Longer transects and steeper gradients should be selected for future connected lakes studies. Nonetheless, the results provided may be typical of a paternoster chain of

lakes. Therefore, chain of lakes should be considered as one unit due to geography, river system and underlying geologic substrate.

Not only was substrate size analyzed in this study, but depth was found to be positively associated with zebra mussel density. Naddafi et al. (2010) studied lakes in Sweden and found substrate and depth to be the best factors predicting zebra mussel density. Depth was not significantly different among lakes, with all four lakes having gradual sloping contours that made sampling along 50-m transects limited in depth variation. Nevertheless, in the current study, the largest densities were found in 2.1-2.2 m of water, keeping in mind water levels slightly fluctuate with seasonality (Bowers and Szalay, 2005). The results I present here are similar to Naddafi et al. (2010) who found quadrats that were placed in 2 m of water had that most zebra mussels associated with each sampling event.

Depths of approximately 2 meters often have ample food supply, larval supply and minimal disturbances that vary dependent on depth gradients (Nadaffi et al. 2010). Although a comparison can be made between depth and zebra mussel density, densities are not static and continue to fluctuate temporally. Nevertheless, depth has been shown to be a good predictor of zebra mussel density in this lake system and should be considered when studying future lake systems.

When I placed the results of this study into a broader geographical context, I suspected there would be similarities with other regional mesotrophic lakes. The Alexandria chain of lakes has had zebra mussels since 2009, and to estimate future

populations, I compared some attributes to Lake Mille Lacs, where zebra mussels have been established since 2005 and has longer-term monitoring data. Granted, Mille Lacs Lake differs in many ways to my study lakes, but there were some similarities that may give managers insight into what could be expected in the years to come.

Mille Lacs is a large lake with a surface area of 53,627 hectares (MN DNR 2014). It has varying substrate from rock and gravel in the southeast region to sand and mud in the northeast region (Jones and Montz, unpublished 2015). Zebra mussels have been increasing in density since naturalizing, from approximately 0.002 per m^2 in 2006 to 13,654 per m^2 in 2012 (Jones and Montz, unpublished 2015). The growth-rate curve suggests a 30-fold population increase annually (Jones and Montz, unpublished 2015). Unlike observations recorded elsewhere, secchi disk readings (*i.e.,* water clarity) did not increase as the zebra mussels proliferated in Lake Mille Lacs. Conversely, all study lakes average secchi depth increased post introduction (Figure 40). In comparison, the four study lakes combined have a total surface area of 11,994 hectares, and are therefore approximately four times smaller than that of Mille Lacs Lake. Another difference is that the study lakes each exceeds the maximum depth of Mille Lacs with a maximum depth of 13 m (MN DNR, 2014).

Mille Lacs Lake also offers many similarities to the study lakes as well. Both areas were highly influenced by glacial activities. Furthermore, the study lakes have comparable TSI values of 47 to 48, indexing both waters as mesotrophic. Both areas are experiencing moderate nutrient loading with 53 ppb total phosphorus in Mille Lacs Lake

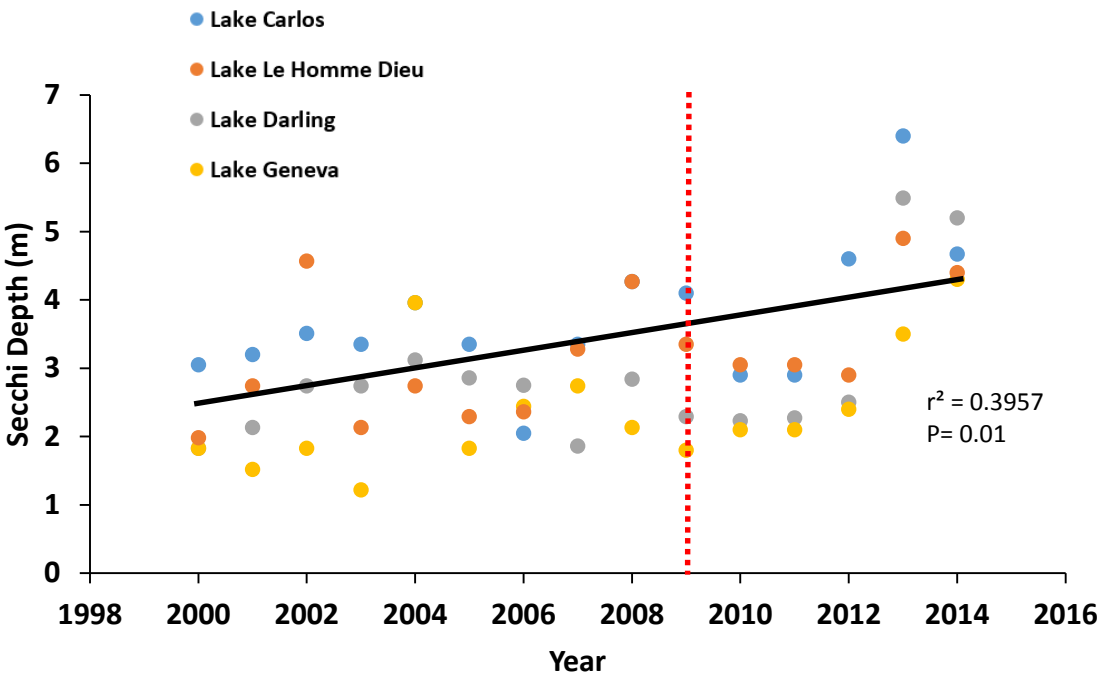

Figure 40. Mean secchi depth in August of each year. Data collected from MN DNR and MPCA. Regression indicates a positive correlation between secchi depth and year. Zebra mussels were first documented in 2009, indicated by the dotted red line.

and an average of 49 ppm into the study lakes. Maximum densities of zebra mussels were seen in Mille Lacs Lake seven years post introduction. Overall, the zebra mussel density seen in this study was 5,331/m^2 (log_{10} = 3.72). If zebra mussel growth is similar to that of Mille Lacs Lake zebra mussels we would expect densities to slow shortly (Figure 41). Moreover, if the similarities between these two waterbodies were enough to make a prediction regarding zebra mussel densities, the study lakes should see their maximum zebra mussel density in the study lakes in either 2016 or early 2017. Nevertheless, considering the differences in depth coupled with density possibly being a good predictor of zebra mussel density in Minnesota, density estimates for study lakes from growth rate curves should be assessed with caution.

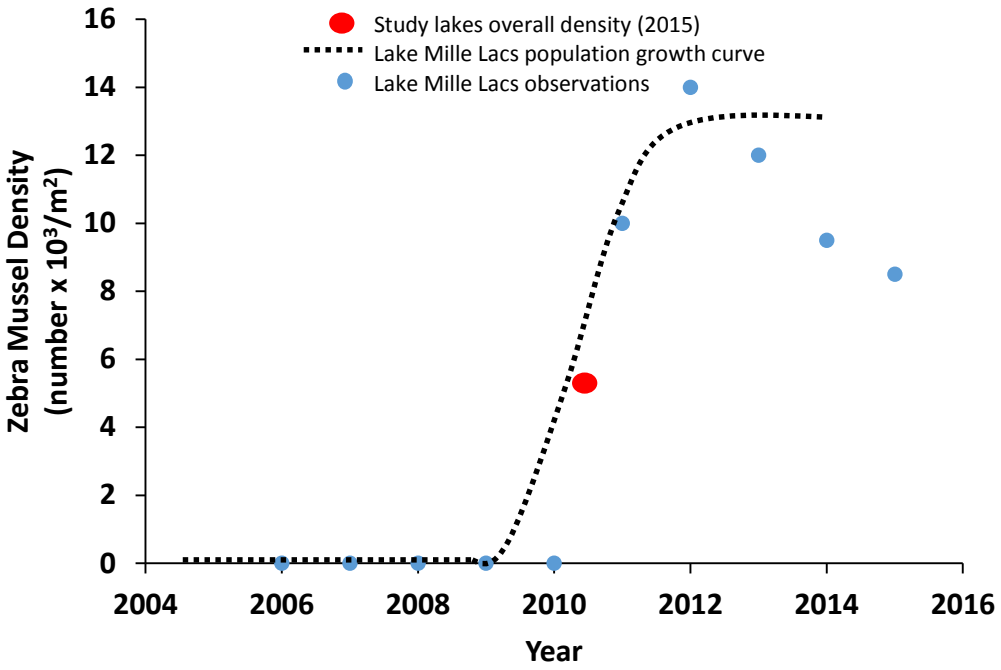

Figure 41. Depiction of the growth curve of zebra mussels in Mille Lacs Lake, Minnesota. Blue dots indicate zebra mussel population density observations in Mille Lacs Lake. The dotted line indicates the modeled growth curve and the red dot indicates the overall zebra mussel density in four west-central Minnesota study lakes. Therefore, if the study lakes follow the same population growth rate, maximum densities could be reached in 2019. Figure modified from Jones and Montz, unpublished data.

Zebra Mussel and Organic Biomass Interactions (Objective 2)

Aquatic plants create alternative opportunities for the attachment of plant-dwelling animals such as zebra mussels (Lewandowski and Ozimek 1997). Organic material can be viable options for settlement by both adult and juveniles. For juvenile zebra mussels, plants may be more important for settlement than other inorganic surfaces. *Chara* spp. grows at high density in many Minnesota lakes and offers ideal refuge from predatory fishes, making it difficult for them to penetrate and reach interior macrophytes (Ozimek 1997). Furthermore, areas with more than 300 g/m^2 of vegetation are difficult for predators to penetrate (Engel 1988). Therefore, areas of high-density biomass may offer protection from predators and disturbances, ultimately yielding more zebra mussels.

Muskó and Bakó (2005) noted the highest zebra mussel densities were located in the areas with the highest density submerged macrophytes. The four study lakes offered approximately 2,431 acres (984 hectares) of vegetation for potential colonization. Although previous work has found that higher densities of aquatic plants were positively correlation with zebra mussel density (Muskó and Bakó 2005), this was not the case for the present study. High plant biomass was not correlated to zebra mussel density. Lake Darling was the only lake that had significance with zebra mussel density but this may have been driven by one outlier with extremely high total macrophyte biomass. Although this was not the situation in the study lakes, is still offers biological evidence of an understudied relationship.

I hypothesized that areas of increased light inhibition would offer as good settling areas, however, zebra mussel negative phototaxic behavior was not observed in the study lakes. Zebra mussel density did not correlate with vegetative cover of aquatic vegetation, most likely due to decomposition of the viable substrate in the winter months. Generally, zebra mussels tend to move away from light and are negatively phototaxic (Kobak, 2000; Toomey et al. 2002). Being negative phototaxic is an advantage to animals, as shade may offer protection from predators and water movement (Toomey et al. 2002). Zebra mussel larvae have been known to avoid bright light and prefer to settle in shaded areas (Marsden and Lansky 2000). Although zebra mussels usually exhibit negative phototaxic behavior, organic substrate offers only ephemeral shading. Dependent on if they are perennial or annual, aquatic plants offer shade for the summer months and then will decompose, making their canopy a less ideal habitat.

Zebra mussels may not show an affinity for areas of shade from aquatic plants, but the plants are still used as attachment substrates. It should be considered that this study was purely observational and other factors may be contribution to their selection of taxa. Furthermore, more sampling quadrats and a closed study could offer a better representation of attachment by zebra mussels. Nevertheless, the total littoral area was 2,431 acres (983 hectares), as previously stated, zebra mussels use *vegetation* differently and do not colonize type equally. Therefore, my hypothesis was rejected because it was observed that zebra mussels showed minimal preference for algaes,

detritus and smaller macrophytes. Overall preference of zebra mussels to differing plant taxa were analyzed as well as juvenile and adult relationships per taxa. Although this has been postulated, to my knowledge, this has not been tested in the field.

In this study, zebra mussel used *Chara* spp. (Figure 42) and filamentous algae similarly for attachment. Furthermore, all transects in the present study were relatively shallow and contained massive amounts of *Chara* spp., a type of microalgae. Connected via a river, the study lakes share similar aquatic taxa and are more similar than that of the headwater lakes (Mäkelä et al. 2004). Similarly, Lewandowski and Ozimek (1997) found *Chara* spp. at 0.5 m in water depth with the highest zebra mussel densities in a Polish Lake. Additionally, Ozimek (1997) found *Characeae* and *Ceratophyllum demersum* to be the dominant plants harboring zebra mussels. Although *Chara* spp. did not harbor the most zebra mussels per biomass, this *vegetation* type was used over other *Potamogeton* spp. and all other macrophytes found in the study. *Chara* spp. was not significantly different than the other algae found in the study lakes, filamentous algae.

Figure 42. Juvenile zebra mussels attached to Chara spp., one of the three types of algaes found in this study. Attachment of zebra mussel on the main axis *stem*.

The attachment of zebra mussels to filamentous algae was a peculiar find (Figure 43). In the current study, more than half of the quadrats sampled had filamentous algae. Attachment was observed both by the byssal interlacing with the filamentous algae as well as the filamentous algae attaching to zebra mussel shells. The attachment of zebra mussels to filamentous algaes was analyzed carefully due to the sheer chance of zebra mussels' ability to get tangled in the algae, mistakenly represented as attached.

To my knowledge, no literature has cited or investigated the likelihood of filamentous algae as a reliable substrate for zebra mussel attachment. A likely explanation for the attachment of zebra mussel to algae is due to their reproduction cycle. Juveniles are created in May or June growing to 80-220 µm in length where they are heavy enough to settle out of the water column (Martel et al. 1995; Kobak 2000). If filamentous algaes is present, and in the study sites it was draped over submerged macrophytes, zebra mussels will settle on top of it and continue its growth. As the zebra mussel grows the filamentous algae is incorporated into the growth of the zebra mussel shell. This would explain the connection of the filamentous algae to the side of zebra mussel shells. I was unable to address this relationship in sufficient detail, but my results indicate that it could represent important habitat and thus, is a good candidate for future work.

Allochthonous material may also offer a good substrate for settling and developing zebra mussels. Furthermore, zebra mussels also used detritus just as

(a)

(b)

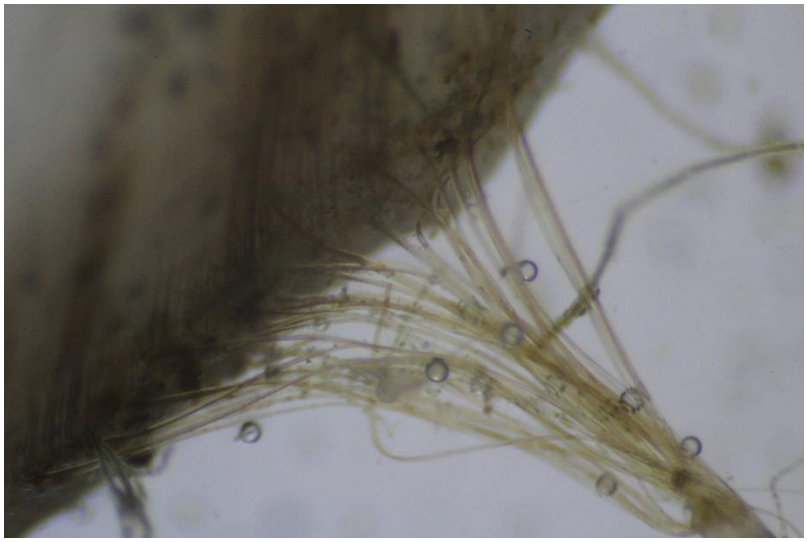

Figure 43. Photograph featuring the zebra mussels' attachment to filamentous algae and the incorporation of algae into zebra mussels' carapace. (a) Filamentous algae wrapped around *Vallisneria americana*. Filamentous algae attached to the zebra mussels' carapace. (b) Microscopic view of the filamentous algaes' attachment to the zebra mussel.

frequently as *Chara* spp. and filamentous algae. With that said, zebra mussels did not choose detritus over any other submerged macrophyte. Nonetheless, detritus offers a good substrate for the settlement of zebra mussels due to the fact that it will stay on the lake bottom until decomposition occurs. Detrital decomposition is controlled by multiple abiotic and biotic conditions and may take a long time (Reshi and Tyub 2007). Allochthonous materials offer zebra mussels an attachments site as well as shade, which is often associated with protection from predation (Toomey et al. 2002). Although the Kruskal-Wallis test indicated no differences among macrophyte taxa, the LEI indicated a few plants that may be of special interest for future research.

In the current study, *Potamogeton* spp. were not used as frequent as both algaes and detritus. This may be due to the homogeneity of algaes in some areas. Nevertheless, zebra mussels do not only prefer alae and detritus according to the LEI. It was found that zebra mussels also may prefer *Potamogeton friesii* and *Najas* spp. over other submerged macrophytes. Although *Potamogeton friesii* would not be an expected substrate for zebra mussel attachment considering its thin morphology it did harbor an average of 11 zebra mussels per unit biomass zebra mussels.

Zebra mussels may prefer this type of pondweed because of its' relative position to the lake bottom. *Najas* spp. would not be expected to be a good substrate considering it is an annual macroalgae but seems to harbor zebra mussels in the study lakes. This may be due to *Najas* spp. ability to form dense beds and offers heavily branched areas for attachment much like *Chara* spp. Most of the species found in this

study were annual plants (*i.e. Chara* spp., filamentous algae, *Najas spp.)* conversely, Ozimek (1997) found the most suitable macrophyte substrates to be perennial, had long-stem stability and were branching.

Other plant taxa found to be viable substrates for zebra mussel attachment were noted by Ozimek (1997) and Lewandowski and Ozimek (1997). The majority of the aquatic taxa with attached zebra mussels included *Ceratophyllum demersum*, *Chara* spp., *Nitellopsis obtuse* and *Stratiotes aloides,* two of which were found in the study lakes (Lewandowski and Ozimek 1997). Although none were seen in the present study, *Myriophyllum spicatum*, another invasive species, has been noted to be good for attachment of zebra mussels (Muskó and Bakó 2005; McComas et al. 2014; Salverson and Zelickson 2015). Considering macrophytes can be used as a substrate and they are easily transported by boaters, understanding macrophyte and zebra mussel relationships can help us better understand potential vectors of AIS spread. Research should focus effort on preference by zebra mussels to taxa, for they can be vectors of transport and well as alternative management applications.

Studies are ongoing at University of Minnesota by Dr. Chun who is addressing the bacterial communities on aquatic macrophytes. Once this is understood in conjunction with zebra mussel preferences to these different taxa, a management strategy may be implemented. If zebra mussel exhibit deterrent behavior to different plant taxa, bacterial communities on these different taxa may be used as biologic control. Considering settling stages of zebra mussels are the most sensitive with a 99%

mortality rates (Mackie and Schloesser, 1996), we may be able to use macrophyte microbial communities to facilitate mortality on settling juveniles.

Furthermore, according to researchers at the U of M long-term future work should include preference for macrophytes and regions of the plant in different lakes (Salverson and Zelickson, 2015). The current study addressed one set of lakes but more lakes in different trophic statuses should be considered. Additionally, future research should address the relationship zebra mussels have with all types of algaes including their ability for attachment. This should be done in a closed laboratory setting to mitigate outside factors and to actually test preference. Although it has been observed that zebra mussels use organic substrates for attachment, juveniles and adults may be using them differently.

In the present study, juvenile zebra mussels were found attached to organic substrates more than adults. Conversely, adults and juveniles seem to use detritus similarly. Juvenile zebra mussels attached more frequently than adults to algaes, Potamogeton spp. as well as all macrophytes. This may be due to juvenile zebra mussel ability to settle on the plants and remain attached until decomposition or the organic substrates were not as available for adults to adhere. With these finding coupled with past research, juvenile settlement on vegetation may be more important than previously thought (Lewandowski and Ozimek, 1997). Although not always homologous, vertical biological substrate is important for the settlement of young zebra mussels (Lewandowski and Ozimek, 1997). Furthermore, juvenile zebra mussels may be utilizing

macrophytes as a temporary substrate for development. This may be due to juvenile zebra mussels' ability to form more temporary byssal threads (produce fewer permanent byssal threads) than adults and can move more readily to a more optimal substrate (Toomey et al. 2002).

According to Lewandowski and Ozimek (1997) annual populations of zebra mussels results from the "numbers dynamics pf planktonic larvae, their mortality and migration". Future research should address larvae mortality and migration as it relates to macrophytes as reliable substrate for recruitment. After over thirty years of research since zebra mussels introduction, there has been an exorbitant amount of research yet, we still don't fully understand the relationships above. Additional research regarding habitat will help to manage this invasive species in Minnesota and other parts of the United States.

Native Mussels in West-Central Minnesota Chain of Lakes (Objective 3)

Study lakes were chosen due to anecdotal evidence of the presence of history native mussels, although these populations were never documented. Native mussel populations have been surveyed extensively in the state by the MN DNR but none in the Alexandria area. Minnesota lake species observed by the MN DNR included the giant floater (*Pyganodon grandis),* fatmucket (*Lampsilis siloquoidea)* and lilliput *(Toxolasma parvum)*. In the current study, the null hypothesis was supported because similar lake taxa were seen in the study lakes as in other Minnesota Lakes, although *Toxolasma parvum* was not observed.

Considering zebra mussels negative impacts on native mussel populations, more research should be done to document their adverse impacts in Minnesota. Furthermore, Unionid mussels cohabitate with *Dreissenid* mussels in their native range with little to no impact on populations. Questions to consider for future research are:

- First, what are the Unionid relationships with *Dreissenid* mussels in their native range?
- Is this relationships purely genetic adaptation based or resistance?
- If adaptation and coevolution is the reason for cohabitation then, can we use these adaptations in our future management of native mussels in the US and Minnesota?
- What other management options are feasible for native mussels in other inland waterbodies pre and post invasion?

Native mussels are very important to the water quality of rivers and lakes thus, future research should address these questions to understand the zebra mussels' role in native mussel population decline in Minnesota.

Conclusion

Zebra mussel are notoriously hard to manage. The species can foul any hard surface, has extremely high fertilization rates with a microscopic larval stage, and as such is one of the most successful invasive species. A major goal of ecological research is to understand the factors that determine the distribution, abundance, and structure of species (Naddafi et al. 2010). Additionally, it is important to understand habitat and selectivity to adequately manage this particular invasive bivalve.

This study showed that zebra mussel density was not a function of underlying geologic substrate in this chain of lake system. Substrate granule size ranges, however, were minimal and did not offer much of a research opportunity. Depth may be a better zebra mussel density predictor in this glaciated area of Minnesota. Although not homogenous, aquatic plants offer attachment for early life stages of zebra mussels. Detritus, algaes (*Chara* spp. and filamentous algae), and smaller macrophytes (*e.g*., *Najas* spp.) were used for attachment over other organic biomass.

Remnant fatmucket and giant floater populations were found in the study lakes, both of which belong to the family Unionidae. Possible questions for future research include the reason for success of Unionid species in their native range in comparison to their introduced ranges. This will help researchers better understand their impacts in these introduced ranges. This study offers a baseline understanding of zebra mussel density in a chain of lake system to compare to other mesotrophic lakes in the state. Lastly, it provides ample future research opportunities to better understand this ecosystem engineer, offering alternative management opportunities.

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Appendices

Appendix 1. Summary of mean parameters observed in the field and abiotic parameters per lake. Vegetation listed were more than 1 % of the total biomass in each lake. Other vegetation was present but in smaller amounts.

	Lake			
Value	Carlos	Darling	Geneva	Le Homme Dieu
Mean Density	6,280	6,455	3,623	5,043
Mean Depth (m)	1.68	1.41	1.37	1.54
Mean Secchi (m)*	4.67	5.20	4.30	4.40
Substrate (Phi value Φ)	-0.19	0.79	0.59	0.60
Mean Biomass (g)	263.67	255.38	323.65	207.66
Mean Percent Cover (%)	50.00	37.50	75.83	58.50
zebra mussel density per biomass (number per g)	6.91	9.65	0.67	5.96
Juvenile density per biomass (number per g)	21.02	17.26	2.35	8.54
Adult density per biomass (number per g)	2.03	2.55	0.16	3.94
Mean filamentous algae biomass (g)	50.23	77.41	63.11	44.69
Mean *Chara* spp. biomass (g)	133.78	140.69	61.57	93.07
Mean algaes (*Chara* spp. and fil. Algae) biomass (g)	184.01	218.10	124.69	137.76
Mean Detritus biomass (g)	26.66	20.58	84.00	26.61
Mean *Ceratophyllum demersum* biomass (g)	1.75	0.51	25.27	1.00
Mean *Najas spp.* biomass (g)	27.11	0.02	9.36	5.17
Mean *Myriophyllum sibiricum* biomass (g)	10.96	0.08	31.56	5.09
Mean *Vallisneria americana* biomass (g)	1.84	5.39	13.00	21.02
Mean *Potamogeton* spp. biomass (g)	10.56	1.36	27.85	18.40
TSI*	43.00	48.00	51.00	56.00
Chlorophyll-*a**	45.00	47.00	52.00	52.00

*denotes data observed by the MPCA

Appendix 2. Appendix 2. Summary table per taxa found in the present study. Vegetation listed were more than 1 % of the total biomass in each lake. Other vegetation was present but in smaller amounts. Expected juvenile and adult estimated were based on juvenile and adults ratios. Expected juvenile and adults zebra mussel counts in table was not normalized (per biomass) but was normalized in the study results. Zebra mussel total and biomass was based on extrapolated per m^2 values.

Taxa	Total per g	zebra mussel Total	Biomass (g)	number Juv Obs (verified)	number adults Obs (verified)	number total Obs (verified)	Juv ratio	Adult ratio	number juv Exp	number adults Exp	number total Exp
Filamentous algae	2.73	19,312.00	7,063.24	1,094.00	632.00	1,726.00	0.63	0.37	12,240.63	7,071.37	19,312.00
Chara spp.	1.91	24,640.00	12,873.36	1,179.00	528.00	1,707.00	0.69	0.31	17,018.49	7,621.51	24,640.00
Ceratophyllum demersum	2.15	1,844.00	855.72	192.00	23.00	215.00	0.89	0.11	1,646.73	197.27	1,844.00
Detritus	1.91	8,976.00	4,708.72	817.00	427.00	1,244.00	0.66	0.34	5,895.01	3,080.99	8,976.00
Najas spp.	2.05	2,596.00	1,267.72	305.00	141.00	446.00	0.68	0.32	1,775.29	820.71	2,596.00
Myriophyllum spicatum	1.22	1,752.00	1,430.80	327.00	70.00	397.00	0.82	0.18	1,443.08	308.92	1,752.00
Vallisneria americana	0.80	988.00	1,237.52	109.00	93.00	202.00	0.54	0.46	533.13	454.87	988.00
Potamogeton spp.	7.43	2,684.00	1,745.04	378.00	145.00	523.00	4.67	1.33	1,953.37	730.63	2,684.00