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Assessing the Short-Term Effects of Translocation on Freshwater Mussels: Is Habitat or Water Quality More Important?

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Assessing the Short-term Effects of Translocation on Freshwater Mussels: Is Habitat or Water Quality More Important?

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Thesis Approval Form

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Abstract

Freshwater mussels (Order: Unionida) are very important to the function of aquatic ecosystems and are typically indicators of good water quality. They provide a valuable link between the water column and the benthic substrate in which they live and are a valuable food resource for many species of animals. However, most species native to North America are currently threatened with extinction, to the point that more than 70% of native freshwater mussels are listed as either threatened or endangered at the state or federal level. The cause of this decline can be attributed to historical over exploitation, habitat alteration, and the introduction of various invasive species that compete with the mussels and their native fish hosts. Translocation is the term used to describe the intentional movement of freshwater mussels to protect and conserve them from anthropogenic impacts. Translocation, however, is only successful if mussels survive. Several studies have found complete mortality of translocated individuals. In addition, this study examines how differences in habitat and water quality at the recipient sites, when compared to the source site, can affect the overall success of the translocation effort. In order to understand the short-term effects of translocation I collected, tagged, and placed 150 Spikes (*Eurynia dilatata*) in one of three experimental sites with varying degrees of similarities and differences in habitat and water quality. Translocation success or failure was evaluated using both survival and growth rates relative to the control (source population). After one year the recipient sites had an average increase in total length of 0.45 mm across the three sites while the source site had an average growth rate of 0.53mm. This difference in growth rate was found to be non-significant ($p>0.05$). From this I conclude that translocation had no effect on mussel growth rates. However, one of the translocation sites experienced high (>50%) mortality over the duration of the experiment while the three other sites, including the source site, experienced low mortality

(<5%) over the duration of the study. Results suggest that water quality vs. substrate played a larger role in survival, and that there may have been variables not assessed (disease vectors) that contributed to higher mortality at one site. In addition, we found that even short-term translocation—i.e., 3 months—gave a strong indication of what sites would be successful vs. unsuccessful. These results have significant management implications. Specifically, (i) the presence of the species at a potential translocation site does not guarantee survival, (ii) short-term (3 month) translocation studies can yield vital information on the potential for success vs. failure, and (iii) the potential to survey and quantify mussel disease vectors is an important consideration about which more research is needed.

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Abbreviations

AWRI	Annis Water Resource Institute
CPOM	Course Particulate Organic Matter
DO	Dissolved Oxygen
FPOM	Fine Particulate Organic Matter
LDB	Left Descending Bank
MDNR	Michigan Department of Natural Resources
PIT	Passive Integrated Transponder
RDB	Right Descending Bank
SRP	Soluble Reactive Phosphate
TP	Total Phosphate
USGS	United States Geological Survey

Chapter 1

Introduction to Freshwater Mussels

Introduction

Freshwater mussels are bivalves belonging to the order Unionida. This order is characterized by its nacreous shells, short syphons, and the parasitic larval life stage (Haag, 2012). While other orders of bivalves are primarily marine, the order Unionida is found only in freshwater, with one exception, which can be found in brackish water (Haag, 2012). There are roughly 1000 species of freshwater bivalves worldwide, with freshwater mussels accounting for roughly 80 percent of this diversity. Freshwater mussels can be found on all continents besides Antarctica (Bogan, 2008).

Even though freshwater mussels are found worldwide, they are not evenly distributed. Australia has 29 species from nine genera while North America, widely considered to be the biodiversity hotspot for freshwater mussels, has roughly 300 species of freshwater mussels representing 53 genera (Bogan, 2008; Williams, et al., 1993). Even in North America the distribution is skewed to the Eastern United States, specifically the Southeast, with the Mississippian Basin and the Gulf Coast Basins with 133 and 147 native species respectively, while the Pacific Coast drainage having only 7 native species (Graf & Cummings, 2007). There is overlap of species between major drainages, so these numbers do not represent endemic species.

Freshwater mussels are considered to be the most imperiled group of animals in North America (Strayer, 2006; Williams et al., 1993). Of the roughly 300 species native to North America, roughly 70% of all species are currently listed as threatened or endangered at either the

state and/or federal level (Williams et al., 1993). Freshwater mussels also have the highest extinction rate of all groups of animals found in freshwater (Ricciardi & Rasmussen, 1999). Ricciardi and Rasmussen (1999), state that the extinction rate for freshwater mussels is roughly 1.2% of species per decade, or roughly 3 species every decade. They predicted that in the future this percentage could climb as high as 6.4% of species per decade going extinct, or roughly 19 species per decade.

The reasons why freshwater mussels are the most imperiled group has changed over the past 150 years, but historical threats have greatly impacted the current distribution and community structure of freshwater mussels today. Historically freshwater mussels were harvested for freshwater pearls and buttons from the mid-1800s to the early 1900s (Haag, 2012; Strayer et al., 2006). Then starting in the early to mid-1900s widescale habitat alteration in the form of river and stream channelization and the installation of new dams greatly altered the flow regimes and habitat in many streams throughout North America which greatly affected the distribution of freshwater mussels (Haag, 2012; Lau et al., 2006; Ligon, et al., 1995; Schoof, 1980; Watters, 1996). Then more recently the introduction of invasive species has also led to the decline of native mussel fauna. The invasive Zebra Mussel (*Dreissena polymorpha*) directly compete with native mussel species, while other invasives such as the Round Goby (*Neogobius melanostomus*) compete with and outcompete native fish that are hosts for native mussel species (Leino & Mensinger, 2017; Balshine, et al, 2005; Schloesser, et al., 1996). The effects of other introduced and invasive species, such as the Asian Clam (*Corbicula fluminea*) is less well studied (Ferreira-Rodríguez, et al, 2018; Haag, 2012).

Freshwater mussels need our help to help conserve the remaining populations and to bolster the wild populations. Freshwater mussels can be difficult to conserve due to their life

history. Since they have a parasitic life stage, there is a need to not only conserve the mussel population itself but also the host species, which is almost exclusively fish, some of which have very specific habitat requirements. This can be as simple as raising awareness about freshwater mussels and their benefits to the public or as complex as reversing historical habitat alterations, i.e. the removal of dams (Haag & Williams, 2014; Lopes-Lima et al., 2018). Haag and Williams (2014) state that the most important goal is to protect and reverse the decline in mussel habitat. In recent years, there has been a rise of mussel propagation and the introduction of these captive reared individuals to bolster wild populations in streams where mussels lived historically.

A useful conservation tool for freshwater mussels is translocation. Translocation is often used when population of mussels are threatened by anthropogenic activities such as, dam removal, bridge removal/repair, or any work that might require instream work that could be detrimental to native mussel beds. In many cases today, if there are listed species present in the work area the state or federal government will require a mussel translocation to take place before any instream work can be done. The mussels are collected and moved to a new area, this could be within the same river or to different states to help reintroduce extirpated species (see Stodola, et al. 2017).

Though translocation is a very useful tool to help conserve and bolster wild stocks of freshwater mussels, there are some drawbacks. It is a labor-intensive process to try and collect all mussels that need to be moved and often times many states require post-translocation monitoring for up to five years post relocation. The act of collecting, exposing to air, possibly tagging, moving, and the placing of freshwater mussels in their new habitat can be quite stressful to the mussels (Ohlman & Pegg, 2019; Wilson, et al., 2011; Waller et al., 1995). This excess stress can lead to some or all of the mussels dying after being translocated. The survival rate of individuals

post translocation can vary widely. In a review of 37 discrete translocation efforts Cope & Waller (1995) found that on average only 50% of individuals will live for five years post translocation. There were several instances where either all mussels survived post translocation, or all mussels died. In many cases where all mussels died there were external factors contributing to higher mortality such as an increase in sedimentation, which buried the translocated individuals such as was observed in Sheehan, et al. (1989).

Purpose

Freshwater mussels are an important native group of animals. They provide a number of valuable ecosystem services including, filtering of water, linking benthic communities to the water column, sediment stabilization, habitat and refugia for fish and macroinvertebrates, and are an important food source for many animals (Vaughn, 2018; Vaughn et al., 2008; Vaughn & Spooner, 2006). They also are indicators of healthy ecosystems and good water quality. However due to over exploitation, habitat alteration, and the introduction of invasive species, freshwater mussel populations have declined rapidly to the point where more than 70% of species are currently listed as threatened or endangered at the state and/or federal level.

A common management tool used to conserve remaining mussel population threatened with extirpation is translocation. Translocation has been used for over 100 years to conserve mussel population (Cope & Waller, 1995). Many previous studies have judged the overall success of translocation by the overall survival rate of the mussels post translocation (see Stodola et al., 2017; Tiemann, 2015; Tiemann et al., 2019). Few of these studies used other factors, such as growth rates, to evaluate mussel health and well-being. This was a major objective of my research project. Specifically, this project looked at how translocation can affect the fitness of the moved mussels. In addition, I also explored how differences in habitat and water quality between

the source and recipient site can affect the overall success of translocation; research that to-date has not been done.

Scope

This study primarily explores the translocation of freshwater mussels in the Thornapple River Watershed. Because of time restraints this project is limited in scope to a one-year translocation effort, instead of the usual five years of monitoring required for most translocation efforts in the state of Michigan. For this study, I focused on adult Spike mussels (*Eurynia dilatata*). Therefore, the application of my results should be limited to adult mussels from species that are considered to be more common and hardy. Due to the size of the streams, I was working in, and the substrate make up, from sand/silt to cobble/boulder, I believe that the results may be applicable to future translocations in similar sized systems in Michigan's lower peninsula, where much of the streams are dominated by sand and gravel. Management recommendations should still be considered on a case-by-case basis as all findings will not be applicable in all situations.

Assumptions

The primary assumption of this study is that attaching passive integrated transponder (PIT) does not affect the overall fitness of the tagged individual due to air exposure and stress from repeated handling. Wilson et al. (2011) found that after tagging, mussels took longer to bury into the substrate when compared to mussels that were not tagged. However, Ohlman & Pegg (2019), found no increase in mortality with the repeated handling and exposure to air. Kurth et al., (2007) found very low rates of mortality, 1.3%, after one year of attaching PIT tags to the outside of the individual's shell.

I assumed that the mortality observed was not attributed to old age of the individuals and was caused by stress related to the translocation process and differences in habitat and/or water quality in this experiment.

For the recipient site named Bend, I assumed that it had nearly identical water nutrient levels as that as observed at Broadway site as it is located 200 meters downstream of the Broadway site and there are no inflows between the two sites that might alter nutrient levels.

Objectives

The primary objectives of this project were to (1) understand the short-term effects of translocation on freshwater mussels and (2) understand how differences in habitat and water quality from the source site of the freshwater mussels to the recipient site can affect the overall success of the translocation effort. With this information, resource managers could better improve the protocols for translocation of freshwater mussels here in Michigan. The primary objective is to better understand the effects of translocation on freshwater mussels, assessed through both growth rates and survivorship/mortality. A secondary objective includes understanding how differences and/or similarities in habitat and water quality can affect the overall success of the translocation effort (i.e., will mussels moved to areas with similar habitat they were taken from have higher growth rates and less mortality than individuals moved to sites with larger differences in available habitat.)

A secondary objective of this project was to raise awareness about the plight of freshwater mussels. Freshwater mussels are a very cryptic group of animals and often times the general public has no awareness of this important group. This has been accomplished through

outreach and further education of members of the public and through publications and presentations of this study.

Significance

Freshwater mussels are the most imperiled group of animals in North America. Translocation is often used to help conserve and save populations of mussels threatened with extirpation. Even though translocation is rather common practice, success varies given the high degree of variation in mortality between different translocation efforts. My study explored how translocation affects the overall fitness of the translocated individuals as well as understanding how habitat and water quality differences can affect the overall success or failure of the translocation effort.

Chapter 2

Assessing the Short-term effects of Translocation of Freshwater Mussels: Is Habitat or Water Quality More Important?

Abstract

Freshwater mussels are vital to aquatic ecosystems and provide important ecosystem services linking the lentic/lotic and benthic communities, stabilizing the substrate, and are a vital food source for many species. A common management practice in the conservation of freshwater mussels is translocation. Translocation is often used when populations of freshwater mussels are threatened with extermination due to anthropogenic effects, such as bridge repair/removal and dam removal. I examine how the act of translocation affects the individuals over the short term (i.e., 1 year), and how differences in habitat and water quality can minimize or exacerbate any short-term effects. In order to do this, I conducted a translocation effort in the Thornapple River Watershed, located in southwest Michigan. For this study, I moved 150 Spike (*Eurynia dilatata*) to three sites with varying degrees of similarities and differences in habitat and water quality. Habitat was judged based off of substrate size, while water quality was judged off of water chemistry and nutrient levels. I found statistically different substrate at both McKeown and Railroad site ($p<0.05$) when compared to the Broadway site. I ranked the sites in the Thornapple River (Railroad) to have different water quality due to nutrient levels being elevated on average when compared to sites in Cedar Creek (Broadway, Bend, and McKeown). All recipient sites, besides McKeown site, had existing populations of Spike. The mussels at the recipient sites were compared to mussels tagged and replaced at the source site, Broadway, for variation in growth rates and mortality. After one year the recipient sites had an average increase in total length of 0.45mm across the three sites while the source site had an average growth rate of 0.53mm. This

difference in growth rate was found to be non-significant ($p>0.05$). From this I conclude that translocation had no effect on mussel growth rates. However, one of the translocation sites experienced high (>50%) mortality over the duration of the experiment while the three other sites, including the source site, experienced low mortality (<5%) over the duration of the study. Results suggest that water quality vs. substrate played a larger role in survival, and that there may have been variables not assessed (disease vectors) that contributed to higher mortality at one site. In addition, we found that even short-term translocation—i.e., 3 months—gave a strong indication of what sites would be successful vs. unsuccessful.

Introduction

North America is widely considered to be the global biodiversity hotspot for freshwater mussels (Williams et al., 1993; Strayer et al., 2006). Roughly 300 species of freshwater mussels inhabit North America, and most are found nowhere else on the planet. North American freshwater mussels have undergone dramatic population declines over the past 150 years and are considered the most imperiled groups of animals in North America (Williams et al., 1993; Strayer et al., 2006). 71% of native mussels are currently listed as endangered, threatened, or a species of special concern at either the state or federal level (Williams et al., 1993). Freshwater mussels have the highest extinction rate of all aquatic organisms of 1.2% of species per decade. This percent is theorized to increase to 6.5% of species per decade (Ricciardi & Rasmussen, 1999). In addition, at least 21 species of North American freshwater mussels have already gone extinct in the last 150 years (Williams et al., 1993). The cause of the decline of freshwater mussel populations has changed over the past 150 years but includes over-exploitation, habitat degradation, and invasive species.

For example, in the late 19th century, mussels were exploited for economic gains. Freshwater mussels were harvested for both freshwater pearls and for mussel shell buttons. The harvest of freshwater mussels often left stretches of rivers devoid of freshwater mussels (Wilson et al., 2011b). In the 20th century, large scale habitat modification further reduced remaining mussel populations. Dams affect water quality and habitat both above and below impoundments. In addition to altering stream habitat, dams also prevent fish migration, which in turn prevents mussel movements, as mussels parasitize fish as larvae and anything that blocks fish therefore also blocks the migration of mussels (Watters, 1996; Katano et al., 2006). Channelization disconnects the river from its floodplain and often results in significant channel alternation in the form of downcutting and habitat niche losses (Duvel et al., 1976; Lau et al., 2006). Stream reaches that are channelized usually consist of one habitat type and are prone to increased flooding, leading to decreased available mussel and fish habitat.

For the past 30 years, invasive species have been one of the biggest threats facing native freshwater mussels. The zebra mussel, *Dreissena polymorpha,* has been a leading factor in the decline of native mussels. Zebra mussels out compete native freshwater mussels by reaching high densities and out filtering, as well as attaching to, native freshwater mussels to the point the native mussel cannot open. In many cases the introduction of zebra mussels to a system has led to the extirpation or near extirpation of all native mussel species (Ricciardi et al., 1998). In addition, round goby, *Neogobius melanostomus,* is also having large impacts on native mussel populations. Round gobies directly compete with native fishes for habitat and food resources leading to a decline in native fish populations, including those that serve as host species for mussels (Balshine et al., 2005; Poos et al., 2010; Burkett & Jude, 2015; Leino & Mensinger, 2017).

Freshwater mussels provide valuable ecosystem services. A single freshwater mussel can filter four liters or more of water a day, and dense beds of millions of mussels are able to effectively filter the entire water column multiple times per day (Vaughn 2018). This improves water clarity and reduces plankton abundance. Mussel waste products, in the form of pseudofeces (unwanted food) and regular feces, provide a vital link for nutrient cycling between the water column and the benthic substrate (Vaughn 2018). Reaches of rivers with higher abundances of freshwater mussels have higher abundances of other macroinvertebrates and can alter fish distribution through habitat modification (Hopper et al., 2019; Vaughn & Spooner, 2006). Acting as "living rocks" freshwater mussels help stabilize benthic substrates, reducing the amount of erosion occurring in the system. Freshwater mussels are also an important food source for many species of animals including, muskrat (*Ondatra zibethicus*), freshwater drum (*Aplodinotus grunniens*), and river otters (*Lontra canadensis*) (Vaughn, 2018).

Habitat improvement and translocation efforts are two of the main conservation practices used with freshwater mussels. Habitat improvement, such as dam removal allows for the reconnection of isolated communities (Strayer, 2008, Haag & Williams, 2014). The removal of dams also facilitates fish movement and allows for colonization of stream reaches previously devoid of mussels (Benson et al., 2018). Translocation, the movement of mussels from one site to another, is often used when populations of mussels are threatened with extirpation due to anthropogenic stream impacts, dam removal, bridge repair, or open trenching for pipelines (Peck et al., 2007; Tiemann et al., 2019). Translocation of freshwater mussels has had varying levels of success throughout its use as a conservation tactic. In some studies, 100% of translocated individuals died after the translocation effort, while in other efforts greater than 80% of mussels survived for 5 years post-translocation (Stodola et al., 2017; Tiemann et al., 2019; Cope &

Waller, 1995). Cope and Waller estimated that in the 37 translocation efforts reviewed, on average about 50% of translocated individuals survived for 5 years after the relocation effort. Their review was done in 1995 and since this time, translocation protocols and management have improved.

Although many studies have examined translocation of freshwater mussels (see Cope & Waller, 1995; Stodola et al., 2017; Tiemann et al., 2019), all have focused on mortality associated with translocation and reintroduction into a new location to measure the overall success or failure of the effort. In this study, I examined additional variables including growth rates, and the varying impacts of habitat and water quality in a short-term (i.e., 3 month and one year) translocation. I hypothesized that freshwater mussels translocated to sites that are more dissimilar in habitat and water quality from the source site would have reduced growth rates and higher mortality rates. I also hypothesized that substrate would have a larger contribution than water quality to these negative effects.

Methods

Study Area

The source site and two of the recipient sites are located in Cedar Creek, a designated cold-water trout stream in Barry County located in south central Michigan (Figure 1). The Cedar Creek watershed drains approximately 118.8 km^2. The watershed is predominantly rural, 83% of the watershed is made up of either agricultural land or forests. An additional study site was located in the Thornapple River, of which Cedar Creek is a tributary. The Thornapple River watershed is home to 19 native mussel species including two Michigan State Threatened Species. It was important for this study to occur within one watershed, to reduce the risk of the inadvertent introduction of invasive species or fish and mussel diseases (see Brian et al, 2021).

Source Site Selection

An initial qualitative freshwater mussel survey of Cedar Creek was conducted to identify a potential source population that could withstand the removal of several hundred individuals. Sites considered were identified from either previous work or were newly surveyed based on access considerations and the potential for mussels to be present. Visual surveys were conducted and if mussels were found, they were identified to species and counted and returned to the substrate. Based on the results of this survey, I selected a source site just downstream of the Broadway Road crossing on Cedar Creek (Broadway). I selected Broadway due to relatively high densities of freshwater mussels, in some places over 20 individuals per square meter.

Receiving (Recipient) sites (translocation) sites

Potential recipient sites (Figure 1, Table 1 & 2) were compared using both quantitative habitat and water quality assessments. The goal was to find sites with varying differences in habitat, water quality, and distance from the Broadway site to see how differences in water quality and habitat can affect the overall success of freshwater mussel translocations. At each potential translocation site selected, I used visual surveys to look for populations of freshwater mussels. If mussels were found, they were identified to species and counted and returned to the substrate. At sites deemed suitable for translocation, further quantitative surveys were conducted. Quantitative habitat and water quality assessments also were conducted at the Broadway site so the potential recipient sites could be compared for similarities and differences. Potential fish hosts were present at all sites but the detection of successful reproduction following translocation was beyond the scope of this project due to time constraints.

Habitat surveys

Wolman pebble counts were used to quantify substrate characteristics (Wolman, 1954). I measured the intermediate axis of 100 randomly selected substrate particles using a ruler while surveying in a zig-zag pattern. Temperature was monitored by recording and logging water temperature every hour for the duration of the study (HOBO Pendant® MX Water Temperature Data Logger**)**. I wanted to select habitat that was suitable for mussel habitation, and preferably have existing mussel beds present.

Water quality

At each site, the water samples were collected in June of 2020 (Table 2) and analyzed for nitrate (NO_3), ammonia (NH_4), soluble reactive phosphate (SRP), total phosphate (TP), sulfates (SO_4), and chlorides (Cl^-). Analyses were conducted by Grand Valley State University (GVSU) Annis Water Resources Institute (AWRI). Orthophosphate (SRP) and Total Phosphate (TP-P) was analyzed using USEPA Method 365.1 Rev 2.0 (1993). Ammonia (NH_3-N) was analyzed using USEPA Method 350.1 Rev. 2.0 (1993). Nitrates (NO_3-N) was analyzed by using USEPA Method 353.2 Rev. 2.0 (1993). Chlorides (Cl^-) and Sulfate (SO4) were analyzed by using USEPA 4500 (1993) Ion Chromatography.

At each site selected for further analysis, I measured dissolved oxygen (DO), conductivity, pH, and temperature using a YSI 650 MDS Multiparameter probe calibrated every two months, except for dissolved oxygen, which was calibrated daily.

Fine Particulate Organic Matter (FPOM) Sampling

Freshwater mussels are filter feeders, consuming organic matter filtered from the water column. Freshwater mussels consume a wide variety of organic matter including different types of plankton, bacteria, and other organic matter. (Haag, 2012). Food preference can also vary from species to species (Tran & Ackerman, 2019). With this in mind, I used fine particulate

organic matter (FPOM) as a way to quantify food resources available to the mussels given that they actively consume FPOM (Christian et al., 2004). I sampled FPOM using a nested net technique; the interior net had a mesh width of one millimeter to exclude course particulate organic matter (CPOM) and the exterior net had a mesh of 0.45 micrometers. Three nets were placed above the translocation grid for 20 minutes. Water velocity was measured at the beginning and end of the 20 minutes. FPOM samples were collected seasonally (October 2020, January, March, and June 2021; Table 2).

Collected samples were brought back to the lab where they were placed in crucibles and dried in a drying oven at 70 degrees Celsius overnight. Dried samples were then weighed on an analytical balance (Ohaus AS2600, precision±0.001 g). Samples were then ashed in a muffle furnace at 550°C for four hours and then weighed again to determine the fraction of organic suspended solids (OSS, dry mass – ash mass) and inorganic suspended solids (ISS, ash mass) in each sample (Hauer & Lamberti, 2017).

Translocation

For this study, I used Spike mussel (*Eurynia dilatata)* for the translocation, as it is the most abundant species at the Broadway site. Spike is an unlisted species and is considered to be secure in Michigan (Hanshue et al., 2019). Spikes are found in a wide range of substrates ranging from sand to cobble (Watters et al., 2009) Freshwater mussels were collected on June 29th, 2020, in accordance with the Michigan Freshwater Mussel Survey Protocols and Relocation Procedures (Hanshue et al., 2019). Mussels were located using snorkel and viewing bucket surveys. Mussels were then identified to species and non-target species were returned to the river substrate. Selected mussels were kept in mesh bags in the stream. The 200 individuals then had Biomark 12mm PIT tags attached to their left hull using super glue and a two-part marine epoxy

to protect the PIT tag as describe by Hartmann et al., (2016). Attaching PIT tags to the outside of the mussels has a high retention rate and has little or no effect on the behavior of the tagged individuals (Wilson et al., 2011, Ashton et al, 2019). PIT tagged mussels were then scanned and ID numbers collected as well as measurements of length, width, height, and mass. Length is the longest axis across one of the valves, width is defined as the total distance along the short axis across one valve, and height is defined as the longest axis across both valves. Once all measurements were taken, the mussels were put back into the bags and returned to the water to allow the epoxy to fully harden for 24 hours. On June 30th of 2020, mussels were then randomly assigned into four groups of 50. One group of 50 was randomly assigned to the Broadway site and the remaining three groups of 50 were randomly assigned to one of the three recipient sites. At each site, the best available mussel habitat was selected. Once the habitat had been identified, a 12m^2 grid was placed on the streambed. Mussels were then randomly assigned to each 1m^2 grid square at a density of five mussels per m^2. Mussels were then hand placed into the substrate, anterior end first.

To track changes in mussel growth and survival rates across the experiment, the PIT tagged mussels were located and collected after three months and again after one year. In order to relocate the translocated mussels, I used a Biomark BP Lite Portable Antennae attached to a Biomark HPR Plus reader. Once located, the mussels were again measured for changes in length, width, height, and mass, and then replaced back into the grid randomly at a density of 5 individuals per square meter. Any dead mussels shells were kept.

Statistical Analysis

Using RStudio, (RStudio Team 2020) I compared the collected habitat and water quality variables. Data was tested for normality using a Shapiro-Wilks test. Data was then tested for

equal variance using a Levene's test. Variables that were normal were then compared using an ANOVA test, and variables that were not normal were compared using a Kruskal-Wallis test. To detect significance between sites, I used a Wilcox sign rank test to look at significance across sites. To calculate the absolute growth rates at each site, I used a simple linear regression at each site starting at zero and plotting the relative change in total length at each of the resurvey periods. An Analysis of Covariance was then used to determine if there was any significant difference in the slopes of the simple linear regression across the sites.

A non-metric multidimensional scaling (NMDS) was used to compare changes in shell dimensions. Environmental factors including, substate size, water chemistry, FPOM were overlayed onto the NMDS. For the NMDS I only used the absolute change length, width, height, and mass for mussels that were found alive as there was no way to determine when moribund mussels had died. I then used an Analysis of Similarity (ANOSIM) post-hoc test to determine if there was any significant difference between sites.

Results

Habitat

From the Wolman pebble count data (Table 3), I found that the dominate substrate at Broadway was sand (<2mm). The dominant substrate at the Bend site was sand (<2mm), the dominate substrate at McKeown site was gravel (24-36mm), and the dominate substrate at the Railroad site was cobble (96-128mm)

A one-way ANOVA was used to compare the variation in substrate size across sites. An ANOVA resulted in a significant difference across sites ($p<2e-16$). A Wilcox rank sign test was run to determine significance comparing sites to Broadway site. There was no significant difference between Bend and Broadway ($p=0.0836$). McKeown ($p=0.0054$) and Railroad

(p=1.3e-11) sites were significantly different from Broadway, having larger substrate sizes. There was also a significant difference in substrate size between McKeown and Railroad site (p=6.5e-09)

Water Quality

The analysis of the nutrients at the sites (Table 4), found that on average, sites within Cedar Creek had similar nutrient levels, while sites in the Thornapple River also had similar water nutrients across sites. The nutrient levels were higher at sites in the Thornapple River when compared to the sites in Cedar Creek.

Fine Particulate Organic Matter

Fine Particulate Organic Matter (FPOM) was sampled in October 2020, and January, April, and June of 2021 (Table 2 & 5). On average, Broadway and Bend both had similar concentrations of FPOM although Bend was slightly higher, possibly due to sampling error. I sampled the upstream site (Broadway) first, then sampled the downstream site (Bend) in the same day. The distance between sites was ca. 200 m, and although a lag-time of 30 minutes occurred, it is still possible that this is an artifact of the sampling approach at this site due to the work at the Broadway site resuspending FPOM and allowing it to flow to the Bend site. The Railroad site, despite being in the Thornapple River, had the lowest FPOM levels.

Translocation site selection

A survey of seven potential translocation sites resulted in the identification and selection of 3 sites that existed along an environmental (physical substrate and water chemistry) gradient that ranged from ‘highly similar’ to ‘moderately similar’ to ‘dissimilar’ with respect to the collection location (Table 1, Figure 1). For habitat sites with no significant difference, I ranked as highly similar, for sites with p values <0.05 I ranked as dissimilar. For water quality, I

considered all sites within Cedar Creek to have similar water quality as they are within the same system and sites in the Thornapple River to have dissimilar as on average the nutrient levels were higher in the Thornapple sites, as well as the other water chemistry parameters (Table 6). Based off my surveys I selected the Bend site as being 'highly' similar for both water quality and substrate. I selected the McKeown site as being 'highly similar' with respect to water quality and 'dissimilar' with respect to substrate. Finally, I selected the Railroad site as being 'dissimilar' in both water quality and substrate.

Three-month resurvey

On September 24th and 25th of 2020, all translocation and source sites were resurveyed. At Broadway, Bend, and McKeown I had high levels of recapture; 49, 50, and 50 individuals, respectively. Of the 50 freshwater mussels recaptured at McKeown, there was one fatality. My third translocation site, Railroad, 45 of 50 individuals were recaptured, of which 12 were dead. All live mussels were measured and weighed, while dead mussels were only measured. All sites had very slight increases in total length, Broadway grew by an average of 0.11mm, Bend by 0.07mm, McKeown by 0.12mm, and Railroad by 0.13mm (Table 7). I observed greater variation in change in width and height across sites but was attribute most of this to human error while measuring the perceived longest axis for width and height. I also observed a non-significant trend for average loss in mass at all sites. I cannot determine if this is loss in body mass from stress or water weight. Dead mussels were not weighed as they varied from fresh dead mussels with the flesh still attached, to empty shells. All live freshwater mussels were replaced back into the grid, while the dead shells were removed.

12 Month Resurvey

On June 1st and June 2nd of 2021, all four sites were resurveyed for the one-year resurvey. At the Broadway site, of the 49 mussels that were found in September of 2020, 48 individuals were recaptured with no mortalities. At the Bend site, of the 50 mussels found in the three-month survey, 47 mussels were relocated with one recorded mortality. At the McKeown site, of the 49 live mussels that remained after the September resurvey, 45 of the individuals were found with no mortalities. At the Railroad site, of the 33 live mussels that were replaced after the September resurvey, 30 mussels were relocated and 15 were found dead. All live mussels were measured and weighed, while dead mussels were only measured. After one year I observed an average change in total length at the Broadway site by 0.53mm, 0.41mm at the Bend site, 0.44mm at the McKeown site, and 0.45mm at the Railroad site (Table 8). The three recipient sites were similar in average growth rate, while the source site, Broadway, exhibited slightly larger growth rates, but this difference was not significant ($p>0.05$). I found a greater variation in width and height similar to the three-month resurvey. I observed a net positive gain in mass at all sites. This might be due to increase in body mass, more water weight, or increased number of attached macroinvertebrates. Dead mussels were not weighed as they varied from fresh dead mussels with the flesh still attached, to empty shells. All live freshwater mussels were replaced back into the translocation sites in case further study of these sites is needed.

Statistical Analysis

For statistical analysis I used only length as I had high variation in both width and height, even when the same person measured the mussels on all three occasions. For the three-month change in length, the data was not normal and was analyzed using a Kruskall-Wallis test, which indicated no significance across sites ($p = 0.5737$). For the one-year change in length, the data

was found to be normal with equal variance. A one-way ANOVA indicated no significant differences sites ($p = 0.541$).

Four simple linear regressions were calculated to predict absolute growth by month at all four sites. All sites had very similar slopes or growth rates, varying from 0.035 to 0.044 mm per month (Figure 2, Table 9). Using an Analysis of Covariance, I determined that there was no significant difference in absolute growth rate measured by total length across sites ($p>0.05$).

In my NMDS of absolute one year growth rates (Figure 3) I found high levels of overlap across the three recipient sites and the source site. From this I can determine that there was no statistical difference in growth rates of all four measurement, total length, width, height, and mass, across the four sites. Environmental data for factors with a p value <0.10 is overlayed on the NMDS (Figure 3). The analysis of similarity resulted in no significant difference between sites ($R=0.03981$)

Discussion

My results are consistent with some previous studies (Bolden & Brown, 2002; Cope & Waller, 1995; Tiemann et al., 2019; Tsakiris et al., 2017) showing that translocation is a viable method for conserving populations of freshwater mussels. I had similar growth rates at the recipient sites when compared to the source site, suggesting that handling, tagging, moving, and monitoring had little effect on growth rates for Spike mussel. The average growth rates across all sites, 0.45 mm, was actually higher than what was reported after one year in Dunn et al. (1999) of -0.4mm after one year. Dunn et al. (1999) is an evaluation of seven different translocation efforts in five different river systems from 1994 to 1997. In 1995, they moved 826 freshwater mussels in the Wolf River in Wisconsin. The Wolf River is a significantly larger river system then the Thornapple River; 225 miles as compared to 88 miles. However, the habitat and

discharge are similar to what I observed at the Railroad site on the Thornapple River. As part of the translocation, they moved 60 tagged Spike mussels. For their tagged Spike mussel, they had only 70% recovery after one year, much lower than 95% reported herein, and they found an average of -0.4mm change in total length compared to an average of 0.45mm after one year. One major difference is that they had zero observed mortality, vs. >50% mortality at the Railroad site.

Two of my recipient sites, Bend and McKeown, had high survival rates one-year post translocation, suggesting that these translocated mussels will survive long-term (Cope & Waller, 1995). The McKeown site, prior to my translocation, had no living freshwater mussels present; old mussel shells were found suggesting that historically mussels may have been present. In order for this site to be considered successful, there needs to be documented reproduction, which will require further surveys. I assume that there will be successful reproduction of translocated individuals at the other three sites as Spike mussel was already present prior to translocation.

The Railroad site, despite a relatively high recovery rate of 45 out of 50 translocated individuals, had 26 observed mortalities. This suggests that site selection, which is one of the goals of this study, is critical in ensuring translocation success (Bolden & Brown, 2002; Cope & Waller, 1995; Dunn et al., 1999).

Possible Reasons for Higher Mortality

I was unable to pinpoint what caused this high level of mortality. I believe that there was adequate space for translocated individuals to bury into the substrate because within a week of translocation, the Thornapple River experienced a storm surge of nearly 800 cubic feet per second (CFS) as compared to summer base flow of 200 CFS, the second highest discharge event in the duration of this study. This data was collected from a USGS gauge located at the Railroad site (USGS 04117500).

If the mussels were not able to successfully bury into the substrate, I would have had much lower recovery rate at the three-month monitoring due to displacement. As several species of freshwater mussels were already present at this site, including Spike mussel, I can safely say that the water quality at the Railroad site was within the requirements needed for freshwater mussels, specifically Spike mussels.

Freshwater mussels as a whole are found in a wide variety of ecosystems from headwaters to the largest of lakes and are found in substrate almost all substrate types, excluding solid bedrock and constantly shifting sands (Coker et al., 1921). Work has been done to understand the microhabitat usage of different species of freshwater mussels, but this is still not well understood, although mussels seem to prefer gravel/cobble substrates (Layzer & Madison, 1995; Strayer & Ralley, 1993). All of the sites, besides McKeown, had existing mussel beds, and had suitable habitat for freshwater mussels (Table 2). From this I can determine that the substrate, at least, was suitable for freshwater mussels.

Due to their life history of being mostly sedentary benthic filter feeders, freshwater mussels are considered to be more sensitive to aquatic pollutants than other aquatic organisms and can be affected by a wide range of organic and inorganic pollutants (Wang et al., 2017). In particular mussels are sensitive to ammonia, which I tested for, and copper. The EC50, the concentration at which 50% mortality occurs, for ammonia and copper can be as low as 1.5mg/L and 10mg/L respectivly (Augspurger et al. , 2003; March et al., 2007; Wang et al., 2017). Glochidia are particully sensitive to polultants such as copper, with the EC50 being less than 0.1mg/L for many species (Gillis et al., 2008). Ammonia levels were much lower than reported lethal levels (range was 0.01 to 0.014 mg/L) (Table 4). Although Cu concentrations were not quantified, I have no reason to believe they would be elevated, although Thornapple Lake is

located approximately 2 river km upstream and there could potentially be legacy contaminants from that source. However Spike mussles were present at the Railroad site, and there was evidence of recruitment of mussels <5 years old (personal observation). The water quality at all of the sites were within acceptible levels for mussels, particularly Spike mussel.

One possible idea is that the mussels translocated to the Railroad site died from thermal stress as this site experienced the highest water temperature of all the sites, 29.72°C. Pandolf et al. (2010) found that temperatures in excess of 30°C could be lethal to glochidia and to a lesser extent juvenile mussel. Given that the mussels that were moved in this translocation were not juveniles (>5 years old) (personal observation) it is unlikely that this increase in temperature is what caused the observed mortality.

Another possible reason for the higher mortality rates observed at the Railroad site is the longer holding time. The Railroad site is the furthest site away, ten road miles, from the source population and therefore was the last translocation site. The mussels destined for the Railroad site were held in an aerated cooler with water while I placed the mussels at the McKeown site. Total time in the cooler was more than an hour but less than two hours. Waller et al. (1995) exposed two species of mussels, Three-ridge (*Amblema plicata*) and Three Horn Wartyback (*Obliquaria reflexa*) to air for four hours and found survival rates of greater than 90%. Stodola et al. (2017), moved federally endangered Clubshell (*Plurobema clava*) and Northern Riffleshell (*Epioblasma triquetra*) from Western Pennsylvania to Eastern Illinois, a trip of roughly eight hours, with the mussels only covered in damp towels. At several of their sites they found similar survival rates as the Bend and McKeown sites (e.g., >90%). I believe that the slightly longer holding time likely did not lead to the higher mortality rate.

Another possible explanation for the higher mortality is predation. Mussels are a common food source for muskrat (Vaughn, 2018), which is found in the watershed. However, I found this to be unlikely given that of the dead shells that were recovered only one shell from the Bend site had evidence of consumption, namely tooth-mark scratches.

A final explanation for the higher mortality rates in the Thornapple River site is disease. The study of freshwater mussel diseases is an up-and-coming field in malacology and is not well understood (Waller & Cope, 2019). If there was a disease or pathogen present at the Railroad site that was not present at the Cedar Creek sites, this could possibly account for the increased mortality. Brian et al. (2021) suggest that this is a major and generally unrecognized concern in translocation projects. In order to know if this was a possibility I would need to disease test mussels from all sites, work beyond the scope of this project, but an extremely interesting and viable avenue for future research.

Conclusions

Studies like this help malacologists better understand why some translocation efforts fail while others succeed. I found that translocation had no apparent effect on the growth rates of the freshwater mussels translocated as part of this study. However, I found high mortality rates at the Railroad site. From the results I conclude that, as long as substrate and water quality are within parameters of mussel habitat that they have minimal to no effect of the growth rates of translocated individuals. I can only speculate on what caused the higher rates of mortality observed at this site. Based on this, I recommend that extensive sampling and monitoring be conducted at all recipient sites prior to translocation to determine if sites are suitable options for mussel placement.

The act of collecting all freshwater mussels from even a relatively small section of a riverbed is labor intensive, and often times requires additional gear such as SCUBA gear. In many cases it is recommended that all translocation efforts have post-effort monitoring to track the success of the translocation effort (see Cope & Waller, 1995; Hanshue et al., 2019). All of this adds up monetarily and translocation and required monitoring efforts are often quite expensive. Studies like this one allow malacologists to better understand habitat and water quality preference for translocated mussels, as well as giving us a better understanding of substrate preference for mussels, in the case of this study Spike mussel. Better understanding the habitat requirements for freshwater mussels is one of the conservation goals laid out by Haag and Williams (2014).

Future Work

Despite the fact that Spike mussel already occurred at the Railroad site, I found high levels (>50%) mortality of translocated mussels at this site. In order for us to understand what might have caused the higher mortality rate at the Railroad site further studies would need to conducted to determine if this site has higher levels of mortality naturally or if the higher mortality rate is only observed in the translocated individuals. Knowing the background mortality rate for the translocation site would allow researchers to know if the mortality observed during the monitoring process is similar to what occurs naturally at the site. Future studies would be needed to track the survivability of the remaining mussel from the translocation to determine if the trend will continue or if the remaining mussel will survive as they have adapted to their new site.

For future studies, to reduce the amount of human error and error associated with monitoring changes in growth and mass, I recommend only measuring length as it seems to be

the measurement that is least subjected to human error; most other published papers only report total length. To reduce the variability found in mass I recommend that the individuals be cleaned of attached macroinvertebrates to remove all weight not associated with the mussel. I would also recommend using buoyant weight technique as described in Molina et al., (2005). This method helps negate the additional weight caused by the water. Molina et al. were then able to determine various different measurements including, total dry weight and shell weight, using species specific weight regression based off of the buoyant mass, although this required sacrificing individuals to allow determination of a variety of metrics.

Management Recommendations

From the results of this study, as well as my review of the literature, I suggest in order for the translocated mussels to have the highest possible chance of survival that potential recipient sites have habitat and water quality parameters similar to that of the site where the mussels originated. I also recommend that potential recipient sites have existing mussel communities similar to that of the mussels being translocated, as the presence of similar mussel community suggests that the recipient site is suitable for long-term mussel habitation. Recipient sites should be located within the same system, assuming favorable water quality and substrate. Locating a recipient site within the same system helps ensure that recipient sites have similar water quality, likely presence of host species, and helps to reduce the risk of incidental introduction of invasive species and mussel-borne diseases (Brian et al., 2021). I also suggest that monitoring should be longer than one year post translocation, preferably three to five years as this would be in accordance with most other studies as well as monitoring protocols here in Michigan (Cope & Waller, 1995; Hanshue et al., 2019; Stodola et al., 2017). Due to the possibility of a disease not being present at the source site but present at recipient site, I recommend, at the minimum

disease testing mussels from both the source site and recipient site, to reduce the chance of disease leading to failure of the translocation effort.

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Tables

Table 1. Summary of sites that were surveyed in the Thornapple Watershed. Sites selected for this translocation experiment are highlighted in grey.

Site #	Site Name	System	Lat.	Long.	Mussels Present?
1	Dam	Thornapple River	42.6063	-85.0969	Yes
2	Boat Launch	Thornapple River	42.6025	-85.0976	Yes
3	Snyder	Thornapple River	42.6079	-85.1176	Yes
4	RR	Thornapple River	42.6159	-85.2365	Yes
5	Spillway	Thornapple River	42.6910	-85.4134	Yes
6	Broadway	Cedar Creek	42.5440	-85.2793	Yes
7	Bend	Cedar Creek	42.5422	-85.2779	Yes
8	McKeown	Cedar Creek	42.5825	-85.2532	Dead Shells

Table 2. Dates for all translocation, monitoring, habitat, water quality, and FPOM sampling

Parameter	Dates
Habitat	6/10-6/12/20
Nutrients	6/4/2020
Water Chemistry (specific conductivity, pH, DO)	5/6/20, 5/13/20, 6/4/20, 6/11/20, 6/30/20, 8/14/20, 9/24/20, 10/16/20, 1/13/21, 3/4/21 4/7/21, 6/1/21
Water Temperature	Hourly Basis 8/1/20-6/1/21
FPOM	10/16/20, 1/13/21, 4/7/21, 6/1/21
Mussel Collection/ Translocation	6/29/20, 6/30/20
Mussel Monitoring	9/24/20, 9/25/20, 6/1/21, 6/2/21

Table 3. Summary table of Wolman pebble counts for each site. Sites selected for translocation highlighted in grey.

Site Name	D16 (mm)	D50 (mm)	D84 (mm)	Dominant Size (mm)	% <2mm	% <6mm
Dam	10	25	45	24-32	12	15
Boat Launch	<2	<2	10	<2	70	74
Snyder	5	20	50	4-6	12	35
Railroad	30	80	150	96-128	3	5
Spillway	10	90	210	128-192	11	12
Broadway	<2	10	30	<2	26	37
McKeown	10	20	45	24-36	11	12
Bend	<2	6	24	<2	39	52

Table 4. Water nutrients for each site. Collected on 6/4/2020. Sites selected for translocation highlighted in grey. Bend site was not surveyed due to being located 200 meters downstream of the Broadway Site, so I assumed nearly identical water nutrient levels.

Site Name	Cl mg/L	S04 mg/L	N03-N mg/L	NH3-N mg/L	SRP-P mg/L	TP-P mg/L
Broadway	11	15	0.2680	0.0109	0.0124	0.0139
McKeown	11	24	0.3058	0.0143	0.0094	0.0235
Dam	25	30	1.1646	0.0499	0.0323	0.0529
Boat Launch	25	30	1.1736	0.0524	0.0224	0.0524
Boat Launch	25	30	1.1604	0.0491	0.0243	0.0493
Snyder	24	29	1.0877	0.0525	0.0296	0.0481
RR	19	26	0.7048	0.0138	0.0100	0.0431
Spillway	20	21	0.9151	<0.010	0.0215	0.0458
Spillway	18	22	0.7066	<0.010	0.0212	0.0451

Table 5. Fine Particulate Organic Matter (FPOM) seasonal results from each of the sites selected for translocation.

Site Name	October 2020 (mg/m^3)	January 2021 (mg/m^3))	April 2021 (mg/m^3))	June 2021 (mg/m^3))
Broadway	1.318	3.386	1.883	1.632
Bend	1.419	3.655	2.720	3.026
McKeown	0.778	1.534	1.689	2.241
RR	0.629	0.824	0.472	0.798

Table 6. Average water quality and water nutrient levels over the duration, May 2020 – June 2021, of the study for sites selected for translocation. Water nutrients were sampled once on 6/4/2020. Temperature was taken on an hourly basis (June 2020-May 2020). DO, pH, and Specific Conductivity was recorded at 12 different times between May 2020 – June 2020

Site Name	Cl (mg/L)	SO4 (mg/L)	NO3-N (mg/L)	NH3-N (mg/L)	SRP-P (mg/L)	TP-P (mg/L)	Avg Temp C	Max Temp C	Min Temp C	DO mg/l	DO%	pH	Sp Cond (µS/cm)
Broadway	11	15	0.172	0.014	0.009	0.024	10.95	25.78	0.68	9.11	90.30	7.79	515.93
Bend	11	15	0.172	0.014	0.009	0.024	10.87	25.60	0.70	8.24	86.13	7.72	520.86
McKeown	11	24	0.289	0.012	0.015	0.033	10.58	22.28	1.99	9.26	93.21	7.86	534.48
RR	19	26	0.7	0.01	0.01	0.043	13.04	29.73	0.60	12.73	131.80	8.55	545.00

Table 7. Summary table of three-month resurvey effort. Table includes average absolute change in mussel dimensions (mm), change in mass, recovery rate, and survival rate. Mass is only of live mussels as dead mussels varied from fresh dead, with flesh still attached, to completely empty shells.

Site Name	# Recovered	% Recovered	# Dead	% Alive of Recovered	Change in Length (mm)	Change in Width (mm)	Change in Height (mm)	Change in Mass (g)	# Returned to Site
Broadway	49	98	0	100.0	0.11 +/- 0.29	0.02 +/- 0.49	0.10 +/- 0.42	-0.58 +/- 0.43	49
Bend	50	100	1	98.0	0.07 +/- 0.26	0.32 +/- 0.53	0.02 +/- 0.51	-1.34 +/- 1.42	49
McKeown	50	100	1	98.0	0.13 +/- 0.41	0.03 +/- 0.60	0.20+/- 0.54	-0.57 +/- 1.69	49
Railroad	45	90	11	75.6	0.12 +/- 0.34	0.04 +/- 0.64	0.05 +/- 0.05	-0.83 +/- 1.56	34

Table 8. Summary table of one year resurvey effort. Table includes average absolute change in mussel dimensions (mm), change in mass, recovery rate, and survival rate. Mass is only of live mussels as dead mussels varied from fresh dead, with flesh still attached, to completely empty shells.

Site Name	# Returned in Sept. 2020	# Recovered	% Recovered	# Dead	% Alive of Recovered	Change in Length (mm)	Change in Width (mm)	Change in Height (mm)	Change in Mass (g)
Broadway	49	48	98.0	0	100.0	0.53 +/- 0.38	0.47 +/- 0.05	0.46 +/- 0.42	1.56 +/- 1.52
Bend	49	47	95.9	1	97.9	0.41 +/- 0.44	0.58 +/- 0.58	0.35 +/- 0.49	0.90 +/- 1.32
McKeown	49	45	91.8	0	100.0	0.44 +/- 0.41	0.46 +/- 0.59	0.50 +/- 0.44	3.35 +/- 2.59
Railroad	34	30	88.2	15	50.0	0.45 +/- 0.30	0.12 +/- 0.73	0.53 +/- 0.59	0.83 +/- 0.84

Table 9. Summary of simple linear regressions based off of the absolute change in total length at all four sites.

Site Name	F-Stat	Degrees of Freedom	p value	Equation	R^2
Broadway	100.7	1 and145	<2.2e-16	Y=-0.011+0.044X	0.41
Bend	54.32	1 and145	1.197e-11	Y=-0.014+0.035X	0.27
McKeown	42.86	1 and143	9.814e-10	Y=0.0077+0.036X	0.23
Railroad	63.41	1 and123	9.523e-13	Y=0.0042+0.038X	0.34

Figures

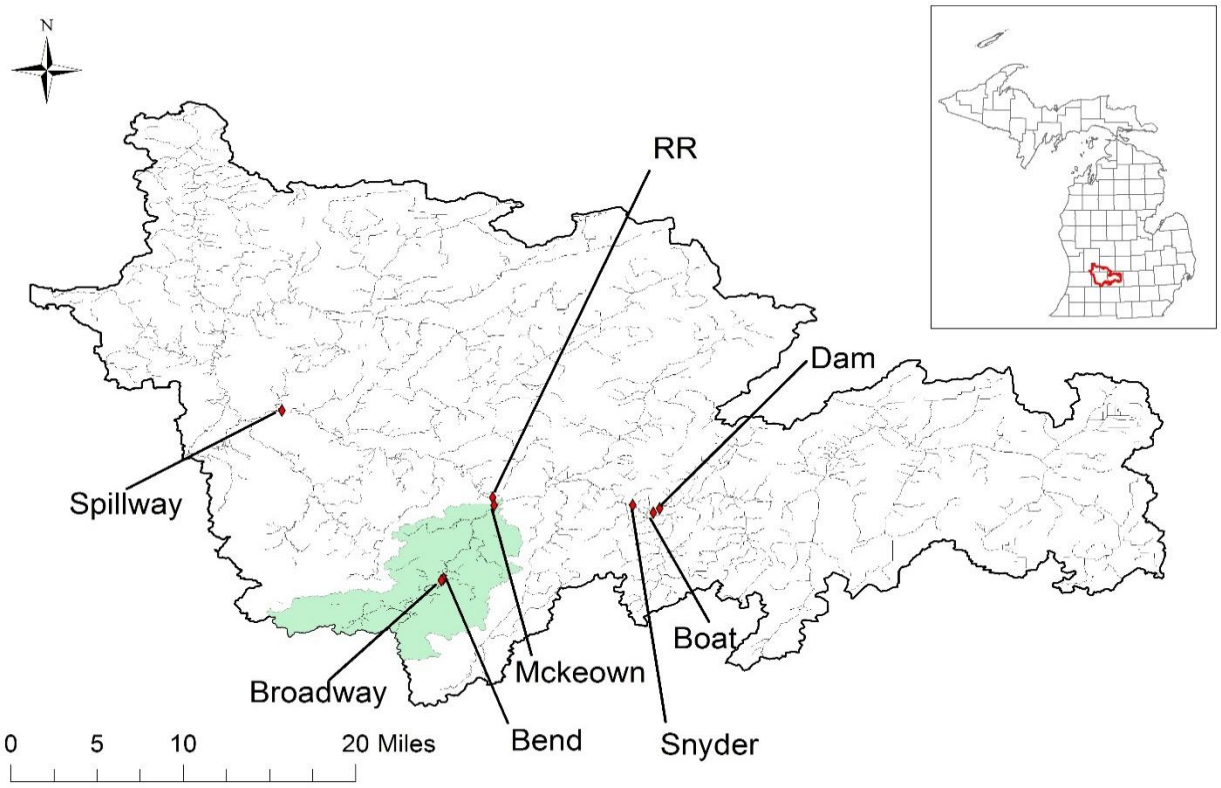

Figure 1. Sites selected for further quantitative analysis in the Thornapple Watershed Michigan, USA. Thornapple River watershed in white and Cedar Creek watershed in green. Source site is Broadway and the selected recipient sites are Bend, McKeown, and Railroad

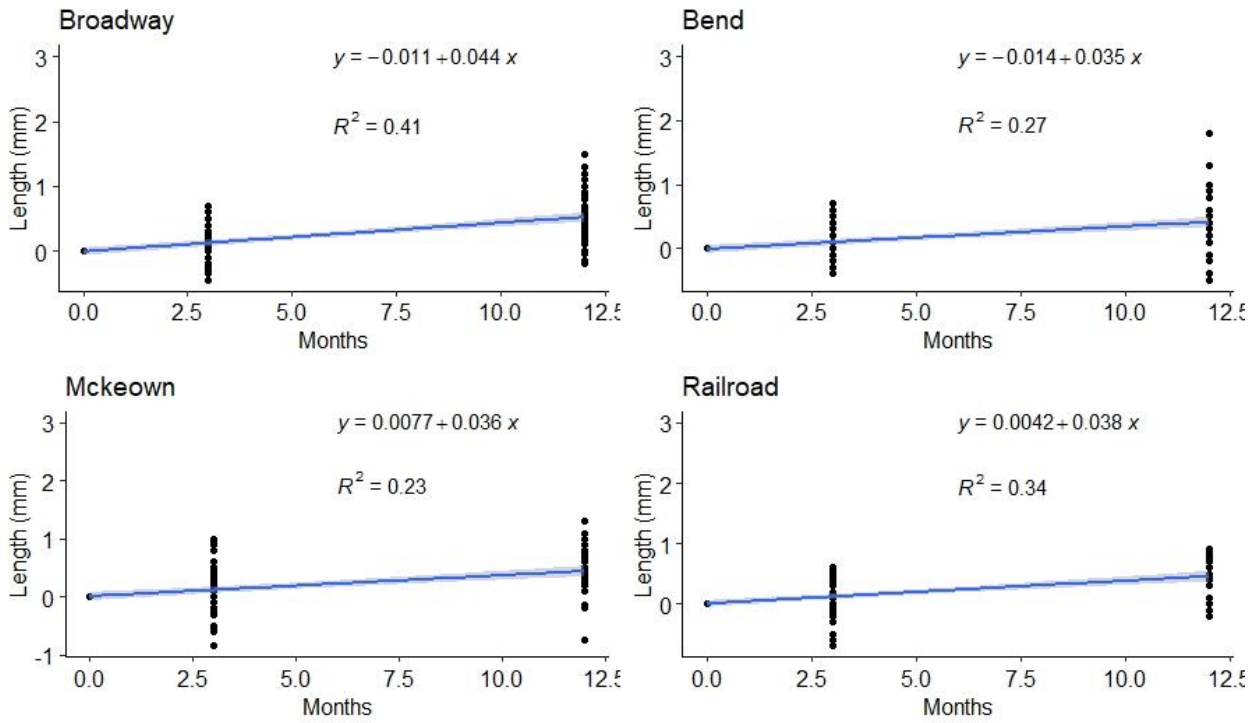

Figure 2. Graphs of the simple linear regression of absolute change in total length after one year at all four sites.

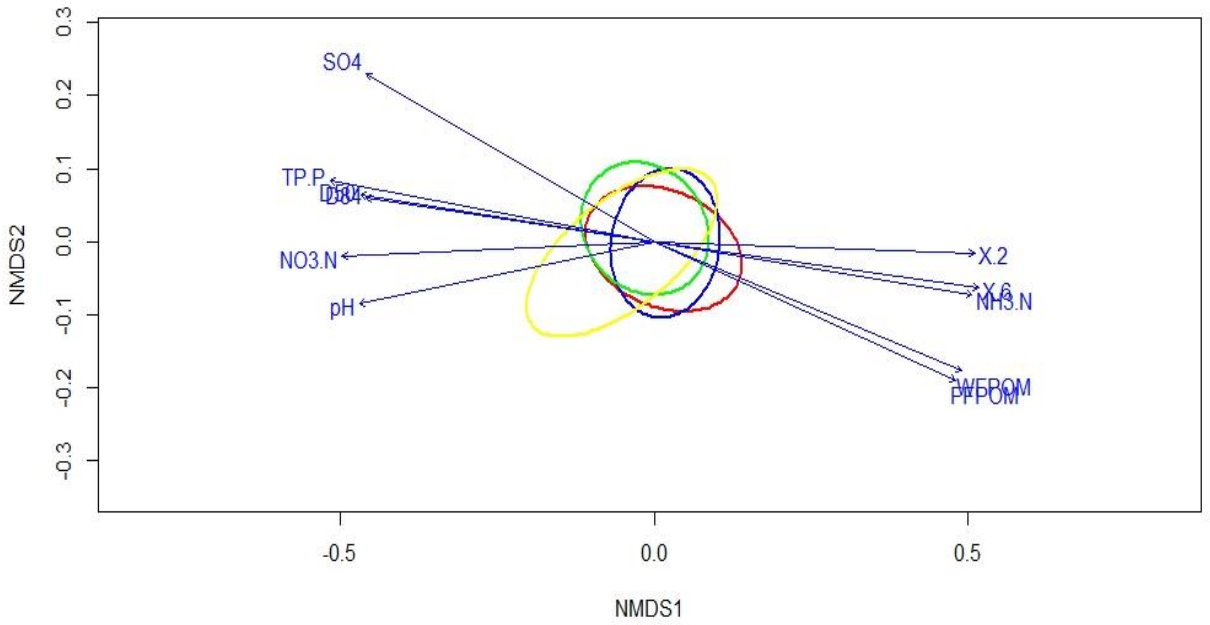

Figure 3. NMDS plot looking at differences in absolute growth rates across sites after one year monitoring. Ellipses are plotted around mussels that fall in one standard deviation from the mean. Broadway is red, Bend is blue, McKeown is yellow, and Railroad is green. Environmental factors with a p-value <0.10 plotted on to the NMDS. Environmental factors are as listed: SO4=Sulfates, TP.P=Total Phosphate, D50=Intermediate width of substrate at 50th percentile, D84=Intermediate width of substrate at 84th percentile, NO3.N=Nitrates, pH=pH, X.2=Percent of substrate less than 2 millimeter, X.6=Percent of substrate less than 6 millimeter, NH3.N=Ammonia, WFPOM=Winter FPOM, FFPOM=Fall FPOM. There is significant overlap between D50 and D84.

Chapter 3

Grand River Fish Community Monitoring Surveys

Introduction

Rivers are some of the most impacted ecosystems in the United States (Citation needed). There are roughly 75,000 dams over one meter in height in the United States and over 300,000 km of streams have been channelized over the years (Graf, 1999; Schoof, 1980). Both dams and channelization greatly alter the function of the rivers by reducing available habitat, altering flow regimes, altering sedimentation rates, and changing water quality and chemistry (Duvel et al., 1976; Ligon et al, 1995). Stream restoration projects are becoming more common. Between 1990 and 2005, the United States spent an estimated $15 billion dollar on stream and river restoration (Bernhardt et al., 2005). However only 10% of these projects, 1.9% in Michigan, were monitored or assessed following project completion (Bernhardt et al., 2005). This lack of monitoring can make it difficult to determine if the restoration project was a success or a failure (Palmer et al., 2005).

In Grand Rapids, Michigan, there is an ongoing project to help to restore the reach of the Grand River that flows through the city. Currently this reach is highly channelized by the presence of flood walls. There are also five run of river dams; the larger 6th street dam, used historically to facilitate the downstream passage for logs, and 4 low-head dams, historically used to maintain river levels to facilitate the downstream movement of sewage. These dams are old and the needs for which they were built no longer exists. The 6th street dam is currently acting as a barrier preventing the upstream migration of the invasive Sea Lamprey (*Petromyzon marinus*). Due to the presence of Sea Lamprey, an adjustable hydraulic barrier is proposed, to be installed

at the upstream end of the restoration reach. This structure can be raised during times of Sea Lamprey migration to prevent them from colonizing the rest of the Grand River Watershed.

Dams act as major barriers to both fish and mussel passage (Katano et al. 2006; Watters, 1996). A fish ladder is present on the 6th street dam, but native fish and smaller bodied fish struggle to use it to pass the dam (Hanshue & Harrington, 2017). The removal of these dams would facilitate the upstream movement of both native migratory fish, introduced salmonids, and native freshwater mussels (Benson et al., 2018; Catalano et al., 2007). Species that would greatly benefit from this project include the Michigan state threatened River Redhorse (*Moxostoma carinatum*), Lake Sturgeon (*Acipenser fulvescens*), and the federally endangered Snuffbox Mussel (*Epioblasma triquetra*).

Methods

Study Site

Fish surveys were conducted in the Grand River in downtown Grand Rapids, Michigan, from July 14th to July 16th of 2020. Discharge in the Grand River went from 3100 to 2750 cubic feet per second over the length of the survey. This reach is defined by a steep slope, 1.04 m/km. This steeper slope translates to faster flows, shallower water, and less fine sediments when compared to the rest of the Grand River. This reach also contains five dams described above.

Three survey reaches were selected from Blue Bridge to Bridge Street (Figure 1) and were located on the right descending bank (RDB). These reaches were established as part of the baseline monitoring for the Grand River restoration project and will be sampled again in subsequent years, both during construction and after the project is completed. In addition, three additional reaches were selected between lowhead dams 2 and 3 (Figure 2), where I sampled

both banks and the center of the channel. This was done in order to survey areas of the river that were too deep to sample with a tote-barge.

Sampling Methods

I used two different electrofishing techniques to accommodate the different depths in the survey reaches and to provide a better estimation of the overall fish community. Catch per unit effort (CPUE) calculations were made to provide a standardized measure for comparisons with future studies. General abundance and species richness were also documented. Electrofishing was conducting in the early morning to prevent excess stress on fish to reduce the amount of mortality due to warmer water temperatures.

In the three monitoring reaches I used a tote-barge electrofishing unit. Captured fish were held in a floating flow through net pen until the reach was finished. At the end of each reach the fish were identified, measured to the nearest millimeter, and released. Due to the large amounts of fish being processed, I only measured the first ten individuals of any given species in each reach. Any capture non-native species were euthanized and removed from the system.

In the reaches between dams 2 and 3 I employed a canoe to sample deeper reaches of the river. The person in the front of the canoe used a smith-root LR-20B electrofishing unit to shock for fish and a net to capture individuals. The person in the back of the canoe was there to keep the canoe straight and back paddled to slow the downstream movement of the canoe. A third person was in kayak to aid and in the hopes of capturing on film any capsizing events. Any fish captured were placed in a water-filled cooler to be processed at the end of each transect. At the end of each reach the fish were identified, measured to the nearest millimeter, and released. Due to the large amounts of fish being processed I only measured the first ten individuals of any

given species in each reach. Any capture non-native species were euthanized and removed from the system.

Results

A total of 807 fish representing 29 species were captured during all electrofishing surveys. The 29 species captured represent a little over a quarter of the 108 species known to exist in the Grand River. These surveys were conducted in the summer, so the vast majority of migratory species were missing from the surveys including Coho and Chinook Salmon (*Oncorhynchus kisutch* and O. tshawytscha), Steelhead (Oncorhynchus mykiss), Brown Trout (Salmo trutta), Longnose Sucker (Catostomus catostomus), and Lake Sturgeon. Of the 29 species captured only the River Redhorse is a species of greatest conservation need and is also a state threatened species. I captured one non-native species, the Round Goby (Neogobius melanostomus). In addition, I capture Common Logperch in several of the transects. These are important as they are considered to be the obligate host for the federally endangered Snuffbox Mussel. The capture data is summarized in Tables 1 and 2.

Nearly 40% of the 807 fish caught in the surveys came from the family Centrarchidae, with the majority being Bluegill (*Lepomis macrochirus).* Cyprinidae represented about one third of the total catch in the surveys with the bulk being made up of Emerald and Sand Shiners (*Notropis atherinoides* and *N. stramineus*).

I had a higher catch per unit of effort using the tote barge than using the canoe electrofishing technique, 8.3 versus 3.6 fish per minute. This could be because I sampled deeper areas with the canoe electrofishing, and was not able to accurately sample and capture individuals from these reaches.

Discussion

In the surveys, I captured nearly one third of species present in the entire Grand River watershed. This could be due to the unique habitat found in the Grand Rapids stretch. This stretch is much more heterogeneous in available habitat and flow patterns while the rest of the Grand River is dominated by slower moving water with sand and silty substrates. This study found representative of both large river species, such as the River Redhorse, and species found in small creeks, for example the Rainbow Darter (*Etheostoma caeruleum*), in this survey. This high level of biodiversity is a good sign for the overall health of the Grand River. Biodiversity is important because it provides greater resistance to disease, invasion of invasive species, and disturbance as compared to systems that are dominated by one or two species (Hooper et al., 2005; Tilman, 1996; Townsend, Scarsbrook, & Dolédec, 1997).

I found 66 Common Logperch (*Percina Caprodes*) over the three survey days. This is important because Common Logperch are considered to be the obligate host for the Snuffbox Mussel. The Snuffbox Mussel is a federally endangered species of freshwater mussel and is one of the target species to benefit from the Grand River restoration project. A sizable Common Logperch population means there is higher likelihood of successful Snuffbox reproduction. In addition, I found 102 Round Goby (*Neogobius melanostomus*), an invasive species found throughout the Great Lakes. Round Goby directly competes with Common Logperch for resources such as food and space (Balshine et al., 2017). This competition can reduce the amounts of Common Logperch and thereby reduce the reproductive success of Snuffbox. The ratio between Logperch and Goby will be an important metric to tract in future monitoring efforts.

The transects along the RDB were previously surveyed in 2018 using similar methods (Preville, 2018). In this survey, they found similar number of species, 30, as compared to the 29 in this survey. Abundances was very similar as well 883 versus 803 in this surveys. Catch per unit of effort was also similar between these two surveys, 9.6 fish per minute as compared to an average of 8.3 fish per minute. There was a difference in family composition collected. In Preville (201p) they found 128 members of the family Catostomidae, the vast majority of these belonging to the genus *Moxostoma.* In this study I found seven members of the family Catostomidae in this tote-barge surveys. This can be attributed to the time of year when these surveys took place.

This survey of the transects in the proposed effort to restore the Grand River in Grand Rapids, Michigan, along with the surveys conducted in Preville (2019) can be used to establish base-line condition of the fish community prior to scheduled restoration efforts in the upcoming years. I do recommend that the surveys be conducted at the same time each year so there is less of a chance of difference in family composition found between this survey and Preville (2018) due to seasonal behaviors of fish. I recommend that these three transects should be resurveyed for the duration of construction and for at least five years post construction to see how the fish community changes as a result of this project. With this and other supporting data, researchers should be able to evaluate the effects of restoration on river ecosystem structure, function, and ecological integrity. Post-restoration monitoring will be important to judge if I can call the Grand River restoration project a success or a failure.

Tables

Table 1. Fish collected in the Grand River in July of 2020 using a tote barge electrofishing unit. Total abundance is broken down by family, Catostomidae, Centrarchidae, Cyprinidae, Gaddidae, Gobiidae, Ichtaluridae, and Percidae. Sites are from downstream to upstream.

Reach	Sample Date	Abundance	Species	CPUE (Fish/Min)	Cato.	Cent.	Cypr.	Gadi.	Gobi.	Icta.	Perc.
Blue Bridge to Pearl Street	7/14/2020	292	17	7.3	0	132	107	1	34	1	16
Gillette Bridge to Dam 3	7/15/2020	190	16	8.6	0	89	67	1	22	0	11
Dam 3 to Bridge Street	7/15/2020	248	23	8.9	7	66	74	0	44	1	56

Table 2. Fish collected in the Grand River in July of 2020 using canoe electrofishing. Total abundance is broken down by family, Catostomidae, Centrarchidae, Cyprinidae, Gobiidae, Lepisosteidae, and Percidae. Catch per unit of effort for total sampling was 3.6 fish per minute.

Transect	Date	Abundance	Species	Cato.	Cent.	Cypr.	Gobi.	Lepi.	Perc.
Right Descending Bank	7/16/2020	13	5	2	0	8	1	0	2
Center Channel	7/16/2020	7	1	7	0	0	0	0	0
Left Descending Bank	7/16/2020	56	14	3	25	21	2	1	4

Figures

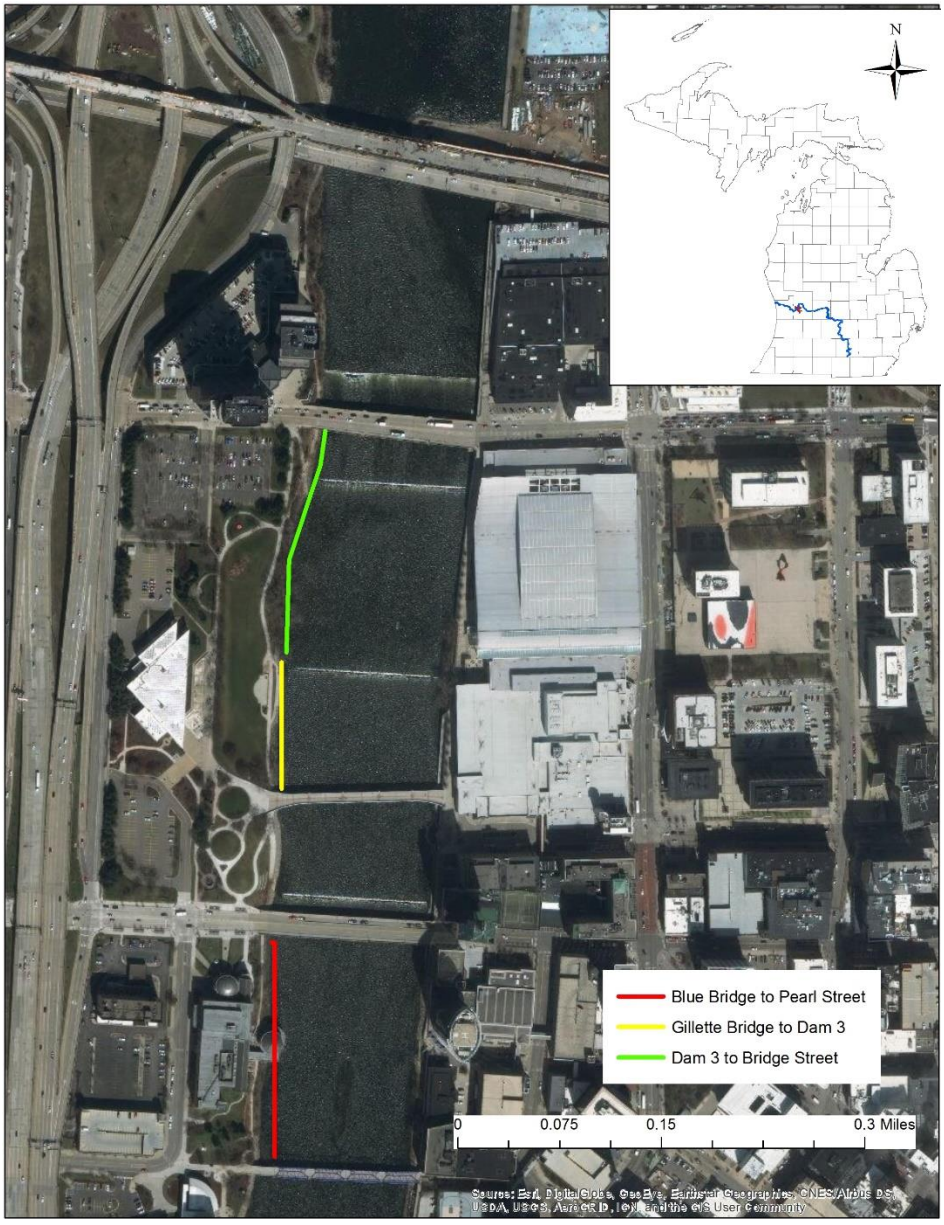

Figure 1: Tote barge electrofishing transects in the Grand River in Grand Rapids. Grand Rapids is highlighted in the inset map of Michigan.

Figure 2: Canoe electrofishing transects in the Grand River in Grand Rapids. Grand Rapids is highlighted in the inset map of Michigan.

Chapter 4

Literature Review and Extended Methodology

Introduction

Freshwater mussels provide valuable ecosystem services. Depending on the species, a single freshwater mussel can filter a four or more liters of water a day, while dense mussel beds are able to filter the entire water column multiple times a day (Vaughn et al., 2008; Vaughn, 2018;). Deposition of nutrients in the form of feces and pseudofeces provides a vital link for nutrient cycling between the water column and the benthic substrate. In stream reaches where freshwater mussels are present, aquatic macroinvertebrate and periphyton densities are higher, possibly owing to the nutrient deposition by mussels (Spooner & Vaughn, 2006). Macroinvertebrates are also known to use both living and dead mussel shells as refugia from stream flow. Freshwater mussels act as "living rocks", helping stabilize benthic substrates and reducing the amount of erosion and downstream sedimentation. In addition, freshwater mussels are an important food source for animals such as, muskrats, *Ondatra zibethicus*, river otters, *Lontra canadensis*, and freshwater drum, *Aplodinotus grunniens* (Neves & Odom, 2016; Vaughn, 2018).

Freshwater mussels are known to exist on every continent but Antarctica with an estimated 1026 species of freshwater mussels in 206 genera and 19 families, worldwide (Bogan, 2008). The greatest mussel biodiversity exists in the Nearctic, or North America (Williams et al., 1993; Strayer et al., 2006). Roughly 300 species of freshwater mussels inhabit the streams and lakes of North America. The highest level of biodiversity is seen in the southeastern region of the United States, with Alabama alone having 178 different species inhabiting its waters. Alabama's mussel diversity is unmatched by any other global biogeographical region, besides the

Indotropics, 219 (Haag, 2012). North America has been described by many as the "rainforest" of mussel diversity, necessitating protection and understanding of these cryptic animals (Haag, 2012).

North American freshwater mussels have undergone dramatic population declines over the past 150 years, making them one the most imperiled groups of animals in North America with 71% of species currently listed as endangered, threatened, or of special concern (Williams et al., 1993). In addition, 21 species of North American freshwater mussels have gone extinct in the last 150 years (Williams et al., 1993). Over-exploitation, habitat degradation, and introduction of invasive species are some of the biggest contributors to the continued decline in freshwater mussel fauna.

Ecology and Life History

North American freshwater mussels have an interesting recruitment strategy, using parasitic larvae, or glochidia to complete their life cycle. All but one species of freshwater mussel uses fish as their host. The salamander mussel, *Simpsonias ambiguia,* uses the common mudpuppy, *Necturus maculosus,* in lieu of a fish host. Glochidia develop by attaching to either the gills or fins of their host species; once attached, they become encysted due to the immune response of the host species. The time it takes for glochidia to mature into a juvenile mussel varies by species and can be anywhere from two weeks to several months (Haag, 2012). Once juvenile mussels drop off their host species, they bury themselves in the substrate where they will spend the rest of their lives.

Different species of freshwater mussels may require different host fish species (Yeager & Saylor, 1995; Barnhart, Haag, & Roston, 2008). Some species of mussels are host specialists,

meaning that only one or two fish species are acceptable. The Snuffbox Mussel, *Epioblasma triquetra*, is an example of a host specialist, and can only use the Common Logperch, *Percina caprodes*, as a host in the wild (Schwalb, Poos, & Ackerman, 2011). The Common Logperch feeds by flipping rocks with its head to uncover macroinvertebrates. When a logperch tries to flip a Snuffbox Mussel thinking it is a rock, it is captured and infected with glochidia. Other species of freshwater mussels are host generalists. Members of the genus *Lampsillis* are examples of host generalist using members of the fish family *Centrarchidae*, black basses and sunfish, as their host. *Lampsillis* glochidia will transform into juveniles on many members of this family.

Broadcast spawning, or the release of glochidia into the water column is a commonly used method to infest host species amongst mussels that lack adaptations to attract them (Barnhart et al., 2008). Species that utilize this method must release larger amounts of glochidia to ensure infection of hosts. The freshwater pearl mussel, *Margaritifera margaritifera,* can produce up to four million glochidia each season. The majority of species that use this method have glochidia specialized for attaching to the fins or skin of their host species (Aldridge & McIvor, 2003)**.** Broadcast of glochidia can be an effective infestation strategy if the host species exists in high densities, proximal to mussel beds. The freshwater pearl mussel uses broadcast spawning to infest salmonids such as Atlantic salmon, *Salmo salar*, possibly taking advantage of mass migrations that increase host species density.

The use of conglutinates, or aggregates of glochidia, is another common strategy to infest host species. Conglutinates are often shaped to look like larval aquatic insects, often mimicking members of the family *Similiidae,* or black flies. Host fish try to eat these “larval insects” and in doing so free the entrapped glochidia, bringing them into contact with their gills. In contrast to broadcast spawning, the use of conglutinates allows mussels to target the feeding guilds of their

host species, meaning fewer glochidia must be produced (Haag & Staton, 2003; Barnhart et al., 2008). Mussels that utilize conglutinates target fish species that feed on larval aquatic insects, such as *Cyprinids.* Members of the genus *Leptodea* will broadcast their glochidia into the surrounding substrate, where it encountered randomly by freshwater drum by suction feeding.

One of the most visible host infestation strategies implemented by freshwater mussels is the use of mantle lures. Mantle lures are modified parts of the mussel's mantle that aim to mimic a prey item such as a minnow or crayfish. Species that use mantle lures are host generalists, using lure types to target different fish hosts or feeding guilds (Haag, 2012). Large lures are often used to target top predators such as black bass, *Micropterus* spp.. Because these top predators are often sight oriented, the mussel will twitch the lures in hopes of attracting a strike. Smaller, cryptic lures are used by some species to target mesopredators such as sunfish (*Lempomis spp.),* or darters (*Percina spp.).* When a host fish strikes the lure the marsupium is ruptured, releasing the glochidia. Glochidia are drawn into the fish's mouth via the negative pressure created by the opening of the fish's buccal cavity and drawn over the gills, resulting in infestation.

Members of the genus *Epioblasma* use host capture as part of their reproductive strategy. Brooding females open their shell valves to expose the mantle, attracting fish. When one of their host species, usually members of the families *Cottidae and Percidae,* touch the mantle, the mussel clamps the shell valves onto the fish, preventing escape. Glochidia are expelled into the gills of a captive fish (Barnhart et al., 2008). Females of *Epioblasmids* have specialized anatomy to facilitate this behavior. Snuffbox Mussels have recurved denticles on the edge of their shells and the northern riffleshell, *Epioblasma rangiana,* have a recurved edge to better hold onto their host species. In some cases if a non-host species is accidently captured its head can be crushed (Barnhart et al., 2008).

One of the strangest host infestations strategies has been dubbed “female sacrifice”. This infestation strategy is observed in species that target freshwater drum. Freshwater drum are large bodied, river fish that are specialized for feeding upon benthic macroinvertebrates such as mussels and aquatic insects. Gravid female mussels will unbury themselves from the substrate and open their shell to attract foraging drum to feed on them. When the drum crushes the mussel with its pharyngeal teeth, glochidia are introduced to the gills, infecting/ infesting them (Haag, 2012). It is also theorized that species specialize in infecting catfish, *Ichtaluridae spp.*, specifically flathead catfish, *Pylodictis olivaris,* utilize similar strategies.

Once the glochidia are attached to a host species they begin to develop into juvenile mussels. The time required for this varies greatly from species to species and is also influenced by water temperature (Barnhart et al., 2008). After the larval mussels develop into juveniles, they drop off and bury themselves in the substrate, maturing into adults. Freshwater mussels are mostly sedentary; however, vertical migrations occur seasonally. Freshwater mussels will occasionally move horizontally over the substrate. On average, freshwater mussels move 11cm +/- 15cm a week (Schwalb & Pusch, 2007). The cause for horizontal movement is not well known at this time (Haag, 2012). Freshwater mussels have been observed moving in order to survive the threat of desiccation, often caused by impoundment drawdown. Newton, Zigler, & Gray (2015) showed that mussels will move more when threatened by desiccation than if no threat existed (Newton, Zigler, & Gray, 2015). Freshwater mussels also display a seasonal vertical migration. Mussels tend to be found on or near the substrate surface in the spring and summer and bury more deeply in the fall and winter (Watters et al., 2009; Schwalb & Pusch 2007). Vertical migration can also be caused by rapid changes in water discharge (Schwalb & Pusch 2007).

Freshwater mussels are often divided into major lineages phylogenetically. Lampsilini is the most recent evolved groups of mussels and often times are the genera that have more specialized infestation strategies and target certain families and/or individuals of fish. For the most part the other major lineages including, Amblemini and Anodontini, are host generalist and for the most part uses broadcast infestation strategy (Barnhart et al., 2008; Campbell et al., 2005; Zanatta & Murphy, 2006). These are some exceptions to this rule, such as members of the genus *Pleurobema* and *Fusconia* use conglutinates and target minnows while belonging to the Pleurobemini lineage.

Study Species

In this study I used Spike mussel as the species of mussel that I studied the effect of translocation on. Spike mussel or just Spike, is an unlisted species in Michigan and is mostly considered to be secure throughout its range. It is found in most states east of the Rocky Mountains and is mostly located in the Great Lakes and the Greater Mississippi drainages. Spike usually have an elongate brown or black shell that is usually purple on the inside but can vary in color. Spikes are considered to be host generalist using species such as, Rock Bass (Ambloplites rupestris), Banded Sculpin (Cottus carolinae), Gizzard Shad (Dorosoma cepedianum), Rainbow Darter (Etheostoma caeruleum), Yellow Perch (Perca flavescens), White Crappie (Pomoxis annularis), Black Crappie (Pomoxis nigromaculatus), Flathead Catfish (Pylodictis olivaris), and Sauger (Sander canadensis). and are thought to broadcast their glochidia as their infestation strategy (Haag, 2012; Watters et al., 2009)

Decline of the North American Freshwater Mussel Fauna

In the late 19th and early 20th centuries, freshwater mussels were over exploited for economic gains. Most freshwater systems are shallow in comparison to marine systems, meaning that they can be easily exploited. For example, in habitats with moderate mussel abundance, 200 mussels per hour can be harvested by hand by a single wading individual (Haag, 2012).

The search for freshwater pearls marked the first major exploitation of North American mussel populations. Pearls are the result of an irritant becoming trapped inside a mussel's shell. The irritant is neutralized by being covered in multiple layers of nacre, encrusting it. All bivalves are capable of producing pearls, but they are most commonly found in mussels. The majority of freshwater mussel produced pearls are small and misshapen, having little or no value. Marketable pearls are sometimes formed; however, they are rare, occurring in less than 0.01% of mussels (Shria, 1913). The rarity of freshwater mussel pearls led to mass depletion of local mussel populations by pearl hunters. These mass exploitations deemed "Pearl rushes", occurred in nearly every state with a sizable mussel population. Some streams were left barren of mussels after the pearl hunters moved through. In some areas of the country, revenue from freshwater pearls exceeded all other natural product industries, besides timber (Claassen, 1994).

In the early "pearl rush" days, empty shells were usually discarded along the river side. This practice quickly changed with the advent of the freshwater shell button industry in the 1890s. manufacturing required the use of the larger heavier shelled mussels to make high quality buttons. The left-over shells from small or thin-shelled species, that often were targeted by pearl hunters had little or no value to the button industry. With the rise of the button industry and the need for larger and heavier shelled mussels put more strain on a growing number of North American mussel species. At the peak of the industry in 1916, 5.75 billion shell buttons were produced with an estimated value of 230 million USD (2009 US$) (Scarpino, 1985).

In the early 20th century, a boom in dam construction for navigation, flood control, and hydroelectric power generation occurred. By the turn of the 21st century, more than 75,000 dams had been built across the United States (Graf, 1999). Dams are detrimental to native mussel populations and affect water quality above and below the impoundments. Above the dam, water velocity is slowed as it enters the impoundment, creating a depositional area, burying any freshwater mussels present. Below the dams, freshwater mussels are subjected to ever changing flow regimes and reduced nutrient availability (Ligon et al., 1995). In addition to altering stream habitat, dams prevent fish migration (Katano et al., 2006). Since mussels rely on host species to complete the reproductive cycle and colonize new stream reaches, impediments to fish movement prevent mussels from moving during their early life stage (Watters, 1996). As dams fragmented mussel communities and impeded fish movements, freshwater mussel populations became isolated. Much of the current mussel biodiversity is found in smaller isolated tributaries, while the main river supports few mussel populations due to conditions in the main river being non suitable for freshwater mussel habitation. Isolated populations are more prone to stochastic events, flooding, and rapid changes in water quality, increasing their risk of extinction (Strayer, 2008). Long distance dispersal throughout these smaller streams is a slow process, due to the life history of the fish that live in these smaller tributaries, as smaller body fish often times have smaller home ranges (Kelly & Rhymer, 2005; Schwalb et al., 2011).

In addition to dams, channelization of rivers in the 20th century had major impacts on lotic habitats and mussel communities. Between 1820 to 1970, 200,000 miles of streams were altered (Schoof, 1980). Channelization causes major shifts in biological communities and hydrological regimes such as, increased water temperature and increased flooding (Duvel et al. , 1976; Lau et al., 2006).

In recent years invasive species have become one of the biggest threats facing native freshwater mussels (Strayer, 2006). Since its introduction to North America in the 1980s, the zebra mussel, *Dreissena polymorpha,* has become a serious threat to native mussel populations. In many cases the introduction of zebra mussels to a system led to the extirpation or near extirpation of all native mussel species (Ricciardi et al., 1998). Zebra mussels attach directly to hard surfaces, including native mussel shells, increasing the amount of energy the native mussel has to exert to bury itself and open to filter feed. Zebra mussels have free living larvae that, unlike native mussel species, do not need a host to complete their life cycle. This reproductive strategy allows zebra mussels to reach densities of 5,000-6,000 individuals per square meter and in some cases >100,000 individuals per square meter (Schloesser et al., 1996). Large populations of zebra mussels are capable of filtering the water column at a high rate, starving native mussels of the nutrients needed to survive.

A more recently introduced invasive fish, the round goby, *Neogobius melanostomus,* is also having a large impact on native mussel populations. Round gobies directly compete with native fishes such as Common Logperch for habitat and food resources and are able to out compete the native fishes (Balshine et alr, 2005; Poos et al, 2010; Burkett & Jude, 2015; Leino & Mensinger, 2017). Common Logperch are the only known host for the federally endangered Snuffbox Mussel, *Epioblasma triquetra,* and a decline in Common Logperch populations would lead to further declines in Snuffbox Mussel populations. Since round gobies occupy the same habitat as Common Logperch, they frequently are infested with glochidia from Snuffbox Mussels, acting as a reproductive sink. The glochidia will not metamorphose into juvenile mussels on round gobies, thus contributing to further declines in snuffbox populations (Tremblay et al., 2016). The effects of other invasive species, black carp, *Mylopharyngodon picues,* Asian

clam, *Corbicula fluminea,* and common carp, *Cyprinis carpio,* are not well known at this time even though the latter two species have coexisted with North American mussels for decades (Haag & Williams, 2014).

With the number of small, isolated populations of freshwater mussels increasing, many conservation biologists are concerned with the minimum viable population size for freshwater mussel communities. A reduction in population size leads to a reduction in individual fitness, known as the Allee effect (Stephens et al., 1999). One of the more common Allee effects on these small, isolated populations is a lack of reproductive success due to a sperm shortage or the inability to find a suitable mate. Eastern elliptio, *Elliptio complanata,* has been observed to have complete fertilization failure once densities drop below 10 individuals per square meter (Downing et al., 1993).

Mussel Community in the Lower Grand River Watershed

In the state of Michigan, there are 43 species of freshwater mussels. Of the 45 species, 19 are currently listed as either endangered or threatened at the state or federal level with an additional eight species listed as species of special concern (Hanshue & Harrington, 2017). The Grand River watershed was historically home to 32 species of freshwater mussel (Hanshue & Harrington, 2017). The Grand River is located in the western region of Michigan's lower peninsula. The Grand River is the largest river in the state, running 406 kilometers, and covering a 14,430 km^2 watershed. The river starts in Somerset Township and drains into Lake Michigan at Grand Haven. The river drops 208 meters from source to outlet.

As in many places around the country, freshwater mussels in the Grand River watershed were exploited for commercial use. During the pearl rushes, many species of mussels were

exploited.. The mucket, *Actinonaias ligamentina,* is a large, heavy shelled mussel that is easy to detect and is rarely completely buried in the substrate. Early freshwater mussel surveys in the Lyons area of the river found that mucket made up 80% of the species collected (Coker et al., 1921). By 1945, muckets made up less than 1% of total freshwater mussels found in the Lyons reach of the river (Van der Schalie, 1948). The dramatic decrease in the Mucket population is attributed to overharvest and unstable flows due to dam construction (Van der Schalie, 1948).

In addition to the freshwater mussel exploitation due to the pearl rushes, the button industry was also active along the river. In 1922, a report by the United States Bureau of Fisheries stated that 4,825,170 pounds of mussel shells were collected from Michigan streams with a value of $196,026. The freshwater mussel harvest was closed in January of 1944 and was to remain closed for five years to allow for mussel populations to rebound after years of exploitation. During this time, the rise of mass plastic production allowed the button industry to shift away from using freshwater mussel shells. The Michigan freshwater mussel season was never opened again. In post-harvest surveys, seven species of mussels that were previously observed in the Grand River were absent (Hanshue & Harrington, 2017). These seven species may have been extirpated or reached below detectable levels.

In addition to commercial harvest, habitat degradation due to large scale alteration of the watershed negatively affected the mussel community in the Grand River. As of 2017, there were 228 regulated dams in the watershed, with many more that are unregulated (Hanshue & Harrington, 2017). The 6th street dam located in Grand Rapids Michigan is an example of a dam that has had serious negative impacts on mussel populations. The dam was constructed in 1866 for hydroelectric power generation and is currently listed as retired. This dam is a major barrier to fish movement due to its height. In 1975 a fish ladder was constructed to allow for upstream

movement of fish, mostly for the introduced Pacific salmons. Native fish however, especially smaller bodied non-game fish, have difficulty or cannot pass through this fish ladder at all and limits upstream migration of both fish and therefor mussels.

Immediately downstream of the 6th street dam are four "beautification" low head dams. These dams were constructed to regulate water level to allow for sewage passage downstream. This series of dams do not block passage for salmon species, but they are theorized to restrict movement of native species during low flow events. These four dams are slated for removal in 2021 allowing for better fish passage in the reach and improved substrate quality for native mussel species, including the federally endangered snuffbox, which currently exists in low densities in this location. After the removal of the low head dams, it is proposed that the 6th street dam will also be removed once an adjustable hydraulic structure is created to prevent the upstream movement of invasive sea lamprey, *Petromyzon marinus*.

Conservation

Freshwater mussels have the highest extinction rate of any aquatic organism in North America; an estimated 1.2% per decade (Ricciardi & Rasmussen, 1999). It is predicted that the extinction rate could reach 6.5%, or roughly 18 species per decade (Ricciardi & Rasmussen, 1999). Roughly 10% of known mussel species have gone extinct in the past 150 years (Williams et al., 1993; Haag & Williams, 2014). There is a great need to conserve the remaining biodiversity of mussels and the valuable role they play in aquatic ecosystems. The main tools useful for mussel conservation include habitat restoration and translocation.

The most important factor for conserving the remaining mussel fauna is habitat improvement (Haag & Williams, 2014). Dams and channelization of the 1900's wreaked havoc

on mussel habitat and aquatic connectivity (Watters, 1996, 1999; Smith & Meyer, 2010). It is commonly known that dams prevent upstream migration of fish and mussels (Watters, 1996). The removal of dams helps facilitate upstream movement of mussels through their host species, connecting isolated populations and reducing the threat of local extirpation due to stochastic events (Catalano et al., 2007; Strayer, 2008).

One of the most prevalent forms of mussel conservation is translocation, or the movement of mussels from one site to another. Translocation is often used when populations of mussels are threatened with extirpation due to anthropogenic stream impacts, dam removal, bridge replacement, or open trenching for pipelines (Peck et al, 2007; Tiemann et al., 2019). As a conservation tactic, translocation of freshwater mussels has experienced varying levels of success throughout its history. In some studies, 100% of translocated individuals died after the translocation effort, while other efforts achieved mussel survival of greater than 80% (Cope & Waller, 1995). Cope et al. (1995), found that a vast majority of sites experiencing high mortality, had limited or no monitoring to evaluate success or failure. Cope and Waller (1995) estimated that in the 37 translocation efforts reviewed, the five-year survival of translocated individuals averaged 50%. Since the 1995 study translocation protocols and management has improved.

The success of translocation is often measured by the proportion of translocated mussels that survive after a period of time. Using survivability as an overall measure of translocation success is complicated by differences in species sensitivity to handling. In addition, some species have much shorter lifespans than other. Stodola et al. (2017), looked at the translocation of northern riffleshell and clubshell, *Plurobema clava,* both federally endangered mussel species. The study found in that mussels relocated to the Salt Fork, a river in Illinois, clubshell had a survival rate of 62%, while the northern riffleshell only had 21% survival after five years. This

could be due to the fact that northern riffleshell are more sensitive to changes and stress and that they have a shorter lifespan, an estimated 12 years. It has been theorized that the majority of individuals that were moved were near the end of their life (Crabtree & Smith, 2009). In Tiemann et al. (2019), mucket had 93% survival and plain pocketbook, *Lampsillis cardium*, had a survival rate of 71% after three years. These species of mussels are less sensitive to changes and stress and have longer lifespans, possibly bolstering the survival rates.

Extended Methodology

Qualitative surveys

Qualitative surveys took place in April and May of 2020. The goal of these surveys was to find areas suitable for mussel translocation. Sites were found by surveying river access sites; boat launches and road crossings. I also canoed a stretch of the river downstream of Nashville Michigan. At sites deemed suitable for mussel habitation, I conducted visual qualitative surveys by either snorkel or view buckets in accordance with Michigan mussel survey protocols (Hanshue et al., 2019). I surveyed for a total of 30 person-minutes per site. All mussels found were collected and identified to species. After the survey, mussels collected were returned to the substrate. At sites where mussels were found, water chemistry (temperature, specific conductivity, dissolved oxygen, and pH) metric were taken using a YSI 650 MDS Multiparameter probe calibrated every two months (pH, SC) or on-site during field surveys (DO). All mussels were collected while in possession of a Michigan Scientific Collectors Permit (#

Tagging Methods

Freshwater mussels used in this study had PIT tags attached to the outside of the shell. Attaching PIT tags has been show to make it easier to recapture tagged mussels in the future (Kurth et al., 2007). Mussels collected for this study were dried on the left valve. A drop of super glue was used to attach the Biomark 12mm PIT tags. The tags were then covered in Devcon (#11800) marine grade epoxy. An epoxy was determine to have the highest retention rate for attaching PIT tags to mussels (Hartmann et al., 2016). This was done to help hold the PIT tags on and to protect the PIT tags. Attaching PIT tags to the outside of the shell is less invasive, leading to lower rates of mortality and has a high retention rate (Kurth et al., 2007). Once the epoxy was applied the mussel was then transferred back to a flow through tank in the river where the epoxy would dry over the next 24 hours. Mussels were held out of the water for roughly 10 minutes during the tagging process. This time out of water was similar to a study done by Ohlman & Pegg (2019) who had the mussels out of water for approximately five minutes anywhere from one time during the year-long? experiment to once a week. They had zero mortality during their experiment. Based off of their findings I assume that no mortality is attributed to handling.

Resurvey Methods

Tagged freshwater mussels were resurveyed on September 24th and 25th of 2020 for the three-month monitoring and again on June 1st and 2nd of 2021 for the one-year monitoring. This was done by having one person using a Biomark HPR plus reader with an attached Biomark BP Lite Portable Antenna to scan the marked 10 m^2 grid. When a tagged mussel was detected another person using a mask and snorkel would dig through the substrate until they found the tagged mussel, which was then collected and placed in a mesh bag and held underwater. This was repeated until all mussels that could be detected within the grid were found and collected. Afterwards, the area around and downstream of the grid was then scanned for any mussels that

may have moved out of the translocation grid. Mussels were then tallied, transferred to an aerated cooler, and individually identified using the HPR plus reader. Length, width, and height were measured for all captured mussels and mass was measured for mussels that were alive. After processing, live mussels were placed in a second aerated cooler. This process took about one minute per mussel. When all mussels were processed, the mussels were returned to the 10 m^2 at five individuals per square meter anterior end first into the substrate.

Appendix

Table 1A. List of freshwater mussel's native to the Thornapple Watershed with state rank

Common Name	Scientific Name	State Rank
Elktoe	Alasmidonta marginata	Species of Concern
Slippershell	Alasmidonta viridis	State Threatened
Cylindrical Papershell	Anodontoides ferussacianus	
White Heelsplitter	Lasmigona complanata	
Creek Heelspitter	Lasmigona compressa	Species of Concern
Flutedshell	Lasmigona costata	Species of Concern
Giant Floater	Pyganodon grandis	
Creeper	Strophitus undulatus	
Three-ridge	Amblema plicata	
Mucket	Actinonaias ligamentina	
Plain Pocketbook	Lampsilis cardium	
Fat Mucket	Lampsilis siliquoidea	
Fragile Papershell	Leptodea fragilis	
Ellipse	Venustaconcha ellipsiformis	Species of Concern
Rainbow	Villosa iris	Species of Concern
Spike	Eurynia dilatata	
Wabash Pigtoe	Fusconia flava	
Round Pigtoe	Pleurobema sintoxia	Species of Concern
Purple Wartyback	Cyclonaias tuberulate	State Threatened

Table 2A. List of all mussels tagged at all sites as part of this study with total measurements of all dimensions and mass taken at all survey efforts. PIT tag numbers are listed as well incase future study on this translocation effort is wanted and/or needed.

		Jun-20				Sep-20					Jun-21				
Site	Mussel ID Number	Length	Width	Height	Mass	Length	Width	Height	Mass	Status	Length	Width	Height	Mass	Status
Broad	3DD.003D99801C	77	40.5	21.45	55.2	77.2	40.5	21.3	53.5	Alive	77.8	40.7	21.8	56	Alive
Broad	3DD.003D998021	77	32.4	19	39.4	77.5	32.4	18.9	38.9	Alive	77.8	33.2	19.3	43	Alive
Broad	3DD.003D998023	75	37	19.6	43.7	75	37.8	20	43.8	Alive	75.3	38.2	20.2	45	Alive
Broad	3DD.003D998024	71.2	34.1	20.25	41.3	71.5	34.1	20.2	39.5	Alive	71.8	35.1	20.6	42	Alive
Broad	3DD.003D998027	69.4	36.1	18.6	33.5	69.5	36	19	33.5	Alive	70	36	19.3	35	Alive
Broad	3DD.003D998028	89.4	45.5	25.5	78.6	89.7	45	25	78.7	Alive	90.2	45.3	25.2	79	Alive
Broad	3DD.003D998029	72	34	19.25	35.5	72	33.9	19.6	35	Alive	71.8	34.5	19.9	37	Alive
Broad	3DD.003D99802D	73.95	36.05	20.3	43	73.5	36.8	20.1	43.2	Alive	73.8	36.7	20.3	47	Alive
Broad	3DD.003D99802E	82	37.4	21.35	50	82.1	37.3	21.1	50.3	Alive	82.1	37.6	21.4	52	Alive
Broad	3DD.003D998033	70.95	36.35	19	38.1	71	36.8	19.2	37.1	Alive	71.1	37.8	19.4	39	Alive
Broad	3DD.003D99803F	83.1	37.5	22	56.9	83.6	36.9	23	55.7	Alive	84.4	38.1	23	58	Alive
Broad	3DD.003D998041	67.2	33	19	34.3	67	33	19	33.4	Alive	67.6	33.3	19.9	36	Alive
Broad	3DD.003D998041	57.95	31.35	15.2	18.8	58.1	31	15	19.5	Alive	58.5	31.9	15.5	19	Alive
Broad	3DD.003D998045	94.6	45.3	25.1	84.8	94.6	44.7	25.1	81.5	Alive	95.5	45.3	25.8	94	Alive
Broad	3DD.003D99804A	67.95	33.5	17	26.1	68.2	33.6	17	26.1	Alive	68.4	33.8	17.3	28	Alive
Broad	3DD.003D99804F	76.35	35.4	30.1	41.4	76.1	36.2	29.9	41.3	Alive	76.6	35.6	29.9	43	Alive
Broad	3DD.003D99805A	74.2	36.35	19	36.6	74.3	35.7	19	37.4	Alive	75	37	19.3	39	Alive
Broad	3DD.003D99805B	66	32.15	17.3	25.3	66.1	31.7	17.5	25.3	Alive	66.1	32.2	18.1	27	Alive
Broad	3DD.003D99805D	84.2	40	23.4	67.7	84.1	41	23.5	66.7	Alive	84.5	41.9	24.2	69	Alive
Broad	3DD.003D99805E	66.55	32.4	17.55	30.5	66.2	33.4	17.1	31.1	Alive	66.8	33.8	17.6	33	Alive
Broad	3DD.003D99805F	89	40.45	22.4	61.7	89.1	40	22.5	60	Alive	89.3	40.6	24	63	Alive
Broad	3DD.003D998061	80.2	39	20.15	47.9	80	39	20.1	46.5	Alive	80.7	39.2	21.1	48	Alive
Broad	3DD.003D998072	62.95	32.3	17.45	24.6	63.1	32	17	25	Alive	63.3	32.5	17.4	25	Alive
Broad	3DD.003D998073	85.3	41.15	21.1	56.9	85.1	41	21.6	55.3	Alive	85.6	41	22	58	Alive
Broad	3DD.003D998088	73.5	36.2	19.1	40.9	73.9	35.5	19.8	40	Alive					Missing
Broad	3DD.003D99808C	80	36.2	25	60.3	80.5	36.4	24.5	59.9	Alive	81.2	37.3	25.2	62	Alive

Broad	3DD.003D998091	79.7	36	22	55.9	80.2	37.1	22.4	55.5	Alive	80.4	37.4	21.8	56	Alive
Broad	3DD.003D998094	80	40	22.5	55.3	80	40.5	22.5	55.1	Alive	80.2	41	22.8	57	Alive
Broad	3DD.003D998096	67.25	32.5	16.4	25.2	67	32	16.5	25.4	Alive	67.8	32.3	17	27	Alive
Broad	3DD.003D998097	89.2	40.2	26.3	77.3	89.1	40.3	26	77.1	Alive	89.5	40.5	26.1	79	Alive
Broad	3DD.003D998098	65.25	30	19.8	30.08	65.5	30	19.9	27.6	Alive	65.9	30.4	20	29	Alive
Broad	3DD.003D9980A1	57.5	28	17.05	20	57.5	29	17.1	20.2	Alive	57.9	29.4	16.9	22	Alive
Broad	3DD.003D9980A3	78.1	39	19	46.5	78.4	38.8	20.5	45	Alive	78.6	39.3	19.5	49	Alive
Broad	3DD.003D9980A6	76.3	37.05	20.5	48.4	76.5	37	20.5	48	Alive	76.9	37.5	21.1	50	Alive
Broad	3DD.003D9980AF	81.3	36.45	22.4	50.8	81	36	22.9	51.5	Alive	81.8	36.7	23.2	54	Alive
Broad	3DD.003D9980B1	64.4	30.25	17	23.6	64.9	30.5	17.2	23.8	Alive	65.9	30.7	17.4	25	Alive
Broad	3DD.003D9980B4	57.2	29	14.15	17.1	57.9	29	13.9	17.7	Alive	58	29.4	14.3	18	Alive
Broad	3DD.003D9980B6	68	32	19.6	30.3	68.5	32.8	19.8	29.5	Alive	69.1	32.4	19.7	32	Alive
Broad	3DD.003D9980BE	69.4	35.35	19.2	38.4	69.4	35	19	38.3	Alive	69.4	35.7	19.1	41	Alive
Broad	3DD.003D9980C6	80.5	40.3	20.05	51.7	81.1	39.5	20	52.3	Alive	81.7	40.7	20.7	54	Alive
Broad	3DD.003D9980CB	76.35	36	19.45	45.5	76	36.2	19.5	42.5	Alive	76.3	36.4	20.3	45	Alive
Broad	3DD.003D9980CD	73.8	34.25	19.4	38.7					Missing					Missing
Broad	3DD.003D9980D1	89	43.2	21	72.2	89	42.8	21.5	70.6	Alive	89.2	43.3	21.6	73	Alive
Broad	3DD.003D9980D2	74.65	35	20.3	40.2	74.4	35.3	21	39.3	Alive	75.5	34.7	21.3	43	Alive
Broad	3DD.003D9980D5	78.2	37.7	20.25	49.9	78.2	37.8	20.5	49.4	Alive	78.9	37.6	20.8	50	Alive
Broad	3DD.003D9980D6	70.5	33.25	18.75	33.6	70.5	33.2	19	31.7	Alive	70.9	33.9	19.1	33	Alive
Broad	3DD.003D9980D7	63	34	12	28	63.4	34	12.8	27.6	Alive	63.4	34.8	13	29	Alive
Broad	3DD.003D9980DA	69.3	36	20.5	37.9	69.9	35.5	20.5	37.4	Alive	70.3	35.8	20.7	39	Alive
Broad	3DD.003D9980DB	74.6	36.3	18.25	48	74.5	36.2	18.7	47.5	Alive	75.5	37	19.5	50	Alive
Broad	3DD.003D9980DF	76.8	34.8	19.05	37.2	76.8	34.9	19.1	36.6	Alive	76.9	34.9	19.9	38	Alive
Bend	3D6.1D5972A470	76	38	20.1	47.9	75.6	38.2	20.1	47.9	Alive	75.8	38.6	20.3	50	Alive
Bend	3D6.1D5972A474	77.3	37.2	19	44.1	77.5	37.5	19.1	41.9	Alive	77.6	38.3	19.1	46	Alive
Bend	3D6.1D5972A49D	78	39.4	18	53.7	77.8	39.1	18.9	53.4	Alive	79	39.9	19.5	56	Alive
Bend	3D6.1D5972A4B4	76	35.5	20.25	43.4	75.9	36	20.1	40	Alive	75.9	36.3	20.4	46	Alive
Bend	3DD.003D99801A	84	40.1	21.2	52.4	84.1	40	20.3	51.5	Alive					Missing
Bend	3DD.003D99801B	74.4	35.5	19.4	40	74.7	35.5	19.2	37.9	Alive	74.8	35.8	19.7	40	Alive
Bend	3DD.003D998025	82.4	41	25	70.9	82.1	41.2	24.8	68	Alive	82.5	41.4	24.7	70	Alive
Bend	3DD.003D99802F	80	40	23	55.5	79.8	40.5	22.6	54.7	Alive	80.1	40.8	22.9	57	Alive

Bend	3DD.003D998031	76	36.1	20.5	47.1	76	35.9	20.1	47.8	Alive	75.9	37.1	20.5	23	Alive
Bend	3DD.003D998032	88	39.7	22	59.1	88	40	21	57.2	Alive	88.4	40.5	21.7	58	Alive
Bend	3DD.003D998036	73.3	37.1	22	50.7	73.1	38	21.9	50	Alive	72.8	38	22.2	52	Alive
Bend	3DD.003D998037	75.5	36.4	18.55	39.8	75.8	35.9	18.8	36.1	Alive	76.3	35.6	19.2	39	Alive
Bend	3DD.003D99803B	84.4	41.1	19.7	70.2	84.6	41.7	20.5	68.7	Alive	84.8	41.9	21	71	Alive
Bend	3DD.003D998040	69.2	36	17	31.8	69.2	36.5	18	30.6	Alive	69.7	36.4	17.6	33	Alive
Bend	3DD.003D998046	90	42	24	65	90	42.8	24.4	59.3	Alive	90.3	41.9	24.4	65	Alive
Bend	3DD.003D998047	79.2	36	19	42.5	79.1	36.4	20	41.6	Alive	79.1	37.2	21		Dead
Bend	3DD.003D99804D	68.4	31	16.6	25.9	68.9	31	16.2	24.7	Alive	70.2	31.2	16.9	28	Alive
Bend	3DD.003D99804E	65.5	32.5	17.4	29.2	65.3	33	17.9	29.4	Alive	65.4	32.6	17.7	29	Alive
Bend	3DD.003D998053	77	35.2	19.4	37.7	77.1	36.5	20.1	37.6	Alive	77.3	36.3	20	40	Alive
Bend	3DD.003D998054	87	41	24.4	71.4	87	41.2	25	69.3	Alive	86.6	41.6	25.3	72	Alive
Bend	3DD.003D998056	86	41	23	58.5	86	40.2	23	57.6	Alive	86.4	41.2	23.1	58	Alive
Bend	3DD.003D998060	74.2	36.3	19.3	44.3	74.6	37.1	18.9	42.2	Alive	74.7	36.2	20.3	44	Alive
Bend	3DD.003D998064	82	40.5	21.1	56.3	81.9	41	21.9	55.2	Alive	81.8	40.4	21.2	57	Alive
Bend	3DD.003D998065	73.6	39	19.1	39.5	74	39.5	19	38.6	Alive	74.5	39.4	20.1	41	Alive
Bend	3DD.003D998067	67.5	34.3	20.7	43.3	68.1	34.9	19.9		Dead					Previously Dead
Bend	3DD.003D998068	62	31.3	16	21.1	62.1	32.1	15.4	22.2	Alive	62.5	33.2	16.7	22	Alive
Bend	3DD.003D998069	75.8	37	20.2	40.3	75.9	38	20	39.8	Alive	76.1	38.3	20.2	41	Alive
Bend	3DD.003D99806E	75	36	19.4	39.4	75	36.9	19.2	40.2	Alive	75.3	36.6	19.2	43	Alive
Bend	3DD.003D99806F	81	36	22.2	56.4	81.1	36.8	22	56.5	Alive	81.8	37.8	23.1	59	Alive
Bend	3DD.003D998074	73.5	33.75	19.4	40.1	74.2	33.5	19.5	40.5	Alive	74.5	33.8	19.4	44	Alive
Bend	3DD.003D998082	83	39.1	22.4	58.8	82.9	39.9	22.8	55.1	Alive	83.8	39.6	22.7	57	Alive
Bend	3DD.003D998083	74.2	35.2	18.55	38.5	74.7	35.3	19	37.5	Alive	75.1	35.3	19.1	39	Alive
Bend	3DD.003D99808B	67	35.3	18.1	34	67	35.9	17.9	33	Alive	67.5	36.1	18.4	34	Alive
Bend	3DD.003D998092	80	38.5	22.3	53	80.4	38.8	22.1	50	Alive	80.9	39	22.3	55	Alive
Bend	3DD.003D99809D	80.3	38.3	22	52.3	80	39.3	21.9	51.5	Alive	80.9	38.9	22.1	53	Alive
Bend	3DD.003D9980A0	75	34.3	19.5	40.7	75	34.3	19.8	39.2	Alive	75.4	34.4	20.2	40	Alive
Bend	3DD.003D9980A5	70.3	36	19.9	38.2	70.1	36.1	19.2	36.3	Alive	70.7	37.8	19.4	40	Alive
Bend	3DD.003D9980A9	74	37	20	42.3	73.8	36.2	19.5	40.3	Alive	74.1	37.7	19.9	42	Alive
Bend	3DD.003D9980AB	92	42.8	23.3	70.6	92	42.8	23.1	67.9	Alive	91.9	42.6	23.7	71	Alive
Bend	3DD.003D9980AC	79	37	20.3	42.7	79.2	36.3	20.1	41.4	Alive	79.4	37.2	20.4	44	Alive

Bend	3DD.003D9980B5	78	40	20.3	43.2	77.6	40.7	20.1	42.8	Alive	78.4	40.7	20.4	44	Alive
Bend	3DD.003D9980BA	81	37.7	19	43.8	81	38.4	20	40.9	Alive	81.8	39	19.6	44	Alive
Bend	3DD.003D9980BD	60.5	28	14.1	18.8	60.9	28.1	14.9	18.5	Alive					Missing
Bend	3DD.003D9980C0	92	43.2	25	79.5	92.1	43.5	24.9	79	Alive	92.3	43.6	25.2	81	Alive
Bend	3DD.003D9980C1	70.1	34	18.1	33.6	70.2	33	17.8	34	Alive	70.3	33.3	17.8	34	Alive
Bend	3DD.003D9980C8	90.3	41.3	24	83.1	90.9	41.9	24	81.7	Alive	91.6	42.3	24.4	84	Alive
Bend	3DD.003D9980C9	83.4	42	20.5	53	83.5	41.9	20.1	51.5	Alive	83.9	42.6	20.6	56	Alive
Bend	3DD.003D9980CA	102	48.3	27.4	107.1	102	48.2	27.8	106.8	Alive	102.2	48.6	27.9	109	Alive
Bend	3DD.003D9980CE	79	36.6	20.5	47	79.2	37.8	20.5	42	Alive	79.9	37.8	21.1	45	Alive
Bend	3DD.003D9980DD	70	36	19.2	37.1	69.9	37	18.8	36	Alive	70.5	36.8	19.1	38	Alive
McKeown	3DD.003D99801D	68	32.5	17.3	28.8	68	33.3	18.3	28.6	Alive	68.1	33.6	18.4	33.2	Alive
McKeown	3DD.003D99801F	73.4	34.5	22	46.8	73.8	33.8	22	45.4	Alive	73.7	33.7	22.5	50.8	Alive
McKeown	3DD.003D998020	57.5	29	19.9	20.7	58.3	29	20	19.9	Alive	58.6	29.5	20.1	23.1	Alive
McKeown	3DD.003D998022	74.2	35.5	17.75	38.7	74.4	35	17.5	39	Alive	74.6	35.8	18.4	42.7	Alive
McKeown	3DD.003D99802A	76.05	36	20.2	44.9	76.4	36.3	20.5	43.5	Alive	76.7	36.2	20.8	47.1	Alive
McKeown	3DD.003D998034	74.65	35.3	20.5	47	75	36.15	21.4	45.3	Alive	75.4	36.6	21.8	39.9	Alive
McKeown	3DD.003D998035	77	35	21.3	45.4	77	35.2	21.6	43.2	Alive	77.3	35.7	21.5	45.9	Alive
McKeown	3DD.003D998039	61.9	28.35	16	23.3	62	29	16.3	21.6	Alive	63.2	28.9	16.3	24.2	Alive
McKeown	3DD.003D99803C	75	36.3	19.4	41.6	75	36.3	19.4	41.4	Alive	75.1	36.4	19.9	46.3	Alive
McKeown	3DD.003D99803D	82	38	22.4	51.5	82.2	38.3	22.5	51.7	Alive	81.8	39.8	23.6	57.2	Alive
McKeown	3DD.003D998043	86	40.1	22	65.6	85.5	40.3	22.2	63.1	Alive	85.8	40.9	22.6	71.5	Alive
McKeown	3DD.003D998048	69.4	36.3	18.3	35.5	70	36	19.2	34.6	Alive	70.3	36.2	20	38.4	Alive
McKeown	3DD.003D998049	72.95	35	19.4	41.1	72.4	34	19	40.3	Alive	72.2	35.1	19.7	42.8	Alive
McKeown	3DD.003D998050	80	37.2	20.25	46.9	80.2	37.5	19.4	46.5	Alive					Missing
McKeown	3DD.003D998059	71	36	21	41.3	71.2	35.3	21	40.7	Alive	71.2	35.6	21.2	44.6	Alive
McKeown	3DD.003D998063	83.1	38.1	22	50.8	84	38.2	22	50.2	Alive	83.8	38.6	22.2	53	Alive
McKeown	3DD.003D99806C	86.05	41.05	22.3	60.5	87	42	22.2	60.1	Alive	86.5	42	22.1	65.1	Alive
McKeown	3DD.003D99806D	79.35	39	22.15	53.6	79.7	38.3	22.45	53.2	Alive	79.7	38.6	22.5	54.2	Alive
McKeown	3DD.003D998078	72.5	38.1	22.2	45.9	72.2	38	22	48.8	Alive	72.8	38.1	22.4	50.1	Alive
McKeown	3DD.003D99807A	78	32	22	48.7	77.7	32.6	22.2	48.2	Alive	77.8	33.1	22.6	52.4	Alive
McKeown	3DD.003D99807B	67	33.3	19.35	37	67.3	32.7	19.6	36.6	Alive	67.7	33.9	20	41.1	Alive
McKeown	3DD.003D99807D	84.15	40	20.7	49.5	83.3	41.3	20.2		Dead					Previously Dead

McKeown	3DD.003D99807E	74.4	37.4	22.2	43	73.8	37.2	20.5	41	Alive					Missing
McKeown	3DD.003D998080	69	33.1	19.1	38.4	69.2	32.7	19	35.7	Alive	69.5	33.5	19.4	39.7	Alive
McKeown	3DD.003D998081	84	40.2	22	65.2	84.4	40	22	60.7	Alive	84.6	40.5	22.2	66.2	Alive
McKeown	3DD.003D998084	71.85	36.6	22.1	50	72.3	36.3	22	50	Alive	72.6	36.4	22.4	53.4	Alive
McKeown	3DD.003D998085	69.4	33.55	18.3	34.3	69.4	34.2	18.3	33.1	Alive	69.7	35.5	18.4	36.8	Alive
McKeown	3DD.003D998086	87.4	42	20.2	57.7	87.4	41.8	21.5	57	Alive	87.8	41.5	20.9	60.6	Alive
McKeown	3DD.003D998087	71.4	33.5	19.05	35.5	71.3	33.7	20	34.8	Alive	71.5	34.2	19.8	36.5	Alive
McKeown	3DD.003D99808E	88.5	42	23	60.1	89.5	42.2	23.1	61.6	Alive	89.5	42.3	23.3	65.1	Alive
McKeown	3DD.003D99808F	78.35	37.3	21.55	50.5	78.6	37	22	50.8	Alive	79.1	38.4	22.3	55.7	Alive
McKeown	3DD.003D998095	77	38.2	20	45.7	77.2	37.4	20.5	44	Alive	77.6	39	20.5	47.9	Alive
McKeown	3DD.003D998099	80.2	37.2	20	40.2	79.6	37.4	20	48.2	Alive	80	37.4	20.2	53.2	Alive
McKeown	3DD.003D99809A	72.15	35.2	20.25	37.4	72.4	36.3	20.5	37.2	Alive	72.4	36	20.8	42.6	Alive
McKeown	3DD.003D99809C	70.5	34.2	19.6	44.6	70.6	35.4	19.4	42.6	Alive	70.9	35.2	19.9	49	Alive
McKeown	3DD.003D9980A7	82.7	39.4	22	58.5	83	39.4	22	56.7	Alive	83.5	39.9	22	62	Alive
McKeown	3DD.003D9980AA	71.85	35.5	18.55	35.5	71	35.3	18.5	34.7	Alive	71.7	35.6	18.8	38.5	Alive
McKeown	3DD.003D9980AD	79	38	19.4	45.6	79.4	37	20.3	44.9	Alive	79.6	38.4	20.3	49.9	Alive
McKeown	3DD.003D9980AE	85.75	40.2	21.3	57.8	85.5	39.4	21.4	56.8	Alive	86	39.9	21.4	62.2	Alive
McKeown	3DD.003D9980B2	85.2	42.15	22.45	62.9	85.6	41.6	22.7	63.4	Alive	86.2	42.4	22.7	68.6	Alive
McKeown	3DD.003D9980B3	78.3	38.5	20.3	51.8	78.1	38.5	21.4	51.2	Alive	78.7	38.1	22.2	57.4	Alive
McKeown	3DD.003D9980B7	76.85	41.4	21.15	58.2	77	41	21.6	58.1	Alive	77.3	41.5	21.5	62.7	Alive
McKeown	3DD.003D9980B9	65.5	32.5	18	30.6	65.5	32	17.3	29.1	Alive	66.6	32.9	18.2	32.1	Alive
McKeown	3DD.003D9980C3	79	37.1	23	55.4	79.4	38	23.1	55.8	Alive	79.5	38.4	23.9	59	Alive
McKeown	3DD.003D9980C4	66.3	31.6	17.45	28	66.4	31.6	18.5	28.4	Alive	66.5	31.9	17.8	30.7	Alive
McKeown	3DD.003D9980C5	67.4	32.2	19.2	35.5	67.7	33.4	20	33.9	Alive	68.3	33.2	18.9	38.7	Alive
McKeown	3DD.003D9980CF	75.5	35.5	21.5	50.4	75.6	35.2	22.2	49.2	Alive					Missing
McKeown	3DD.003D9980D4	61	29.6	14.4	19.3	61.5	30	15	19.6	Alive	61.7	30.1	15.2	23	Alive
McKeown	3DD.003D9980D8	76	37	22.7	51.8	76	37	23	51	Alive					Missing
McKeown	3DD.003D9980DC	65	33.3	17	28.6	65	33.1	17.4	28.7	Alive	65.2	34	17.5	31.5	Alive
Railroad	3DD.003D998019	61.15	31.5	16.8	24.8	61.5	31	15.5	20.2	Alive	61.9	31	17.5		Dead
Railroad	3DD.003D99801E	68.3	33.55	17.45	29.4	68.5	34	17.4	29.8	Alive	69	34	18.4	30.6	Alive
Railroad	3DD.003D998026	82	38.3	23.3	54.1	82	38	23.5		Dead					Previously Dead
Railroad	3DD.003D99802B	77.5	38.8	22	47.9	77.5	38.3	22.4		Dead					Previously Dead

Railroad	3DD.003D99802C	70.5	36	19	33					Missing					Missing
Railroad	3DD.003D998030	66.6	32	16.4	24.8	66.7	32	17	24.8	Alive	67.1	32.3	17.3	26.4	Alive
Railroad	3DD.003D99803A	75.25	37	21	45.3	75.2	36.4	21		Dead					Previously Dead
Railroad	3DD.003D99803E	89	42.2	24	69.6	89.2	42	24.1	69.7	Alive	89.3	42	25		Dead
Railroad	3DD.003D998042	78	39.3	21.3	47	78	39.4	21.1		Dead					Previously Dead
Railroad	3DD.003D998044	80.2	42.2	22	57.6	80.3	41.3	22	55.8	Alive					Missing
Railroad	3DD.003D99804B	79.2	35	20.5	47.1	79	35.2	22		Dead					Previously Dead
Railroad	3DD.003D99804C	67.2	32.5	17.5	32.7	67.2	33.1	18.3	31.1	Alive	67.1	33.1	17.4		Dead
Railroad	3DD.003D998051	69	34.2	18	33.2	69.5	35	17.5	33	Alive	69.7	34.7	17.7	33.7	Alive
Railroad	3DD.003D998052	88.05	41	22.6	64.5	88	41.3	23.3		Dead					Previously Dead
Railroad	3DD.003D998055	69.7	33.3	17.2	30.1	69.6	34	17	29.3	Alive	69.5	34.2	17.7	31.1	Alive
Railroad	3DD.003D998057	73	34.6	17.2	30.6	72.5	35	17.4	30.9	Alive	73	34.9	17.5	31.4	Alive
Railroad	3DD.003D998058	82	40.5	19.4	50	82.4	40	19.4	50.3	Alive	82	40	19.9	52.5	Alive
Railroad	3DD.003D99805C	79.7	35	22	46.5	79.1	35.3	22	46.6	Alive	79.7	35	23.1		Dead
Railroad	3DD.003D998062	80	38.2	24.2	66.3	80.3	38	24.2	64.5	Alive	80.5	37.9	25		Dead
Railroad	3DD.003D998066	80.1	37.8	19.1	40.9	80	38.3	19	38.2	Alive					Alive
Railroad	3DD.003D99806A	72.4	34.2	17.4	30.1	73	34.1	17	30.4	Alive	73.3	35	18	30.4	Alive
Railroad	3DD.003D99806B	80	39.2	20.5	51					Missing					Missing
Railroad	3DD.003D998070	70	36	19.4	35.9	70	35.5	19.2	35.7	Alive	70.7	35.8	20		Dead
Railroad	3DD.003D998071	70.15	34	19	35.9	70	35	19	33.7	Alive	70.6	34	19.1		Dead
Railroad	3DD.003D998075	73.3	37	20.5	43.2	73.6	36.6	21	42.8	Alive	73.6	37.4	21.3	43.5	Alive
Railroad	3DD.003D998076	65	19.1	16.3	19					Missing					Missing
Railroad	3DD.003D998077	70.2	33.35	19.4	34	70.8	34	19.4	34.4	Alive	71	34.5	19.6	34.7	Alive
Railroad	3DD.003D998079	75.4	34.4	18.3	37	75.3	34.2	19		Dead					Previously Dead
Railroad	3DD.003D99807C	81.4	40.25	19.7	50.8	81.5	41	20	49.7	Alive	81.8	40.8	21	53	Alive
Railroad	3DD.003D99807F	80.4	37.25	21.15	50.7	81	37.5	21.35	50.8	Alive					Alive
Railroad	3DD.003D99808A	88	42	25	72.6	88.2	41.6	25	72.4	Alive	88.3	42.2	25.3	72.4	Alive
Railroad	3DD.003D99808D	71.2	35	19	33.2	71.6	34.4	18.5	32.2	Alive					Alive
Railroad	3DD.003D998090	77.7	39	23.1	53.5	77.5	38.5	22.5	51.4	Alive	78.3	38.3	23.5	52.9	Alive
Railroad	3DD.003D998093	77	38.1	21.35	51.9	77.6	37.5	21.3	51.5	Alive	77.7	39.8	21.9		Dead
Railroad	3DD.003D99809E	79.5	37.4	20	47.1	80	37	20	47.8	Alive	80	36	20.2	48.4	Alive
Railroad	3DD.003D99809F	72.7	32.6	17.2	30.9	72.4	32.4	18		Dead					Previously Dead

Railroad	3DD.003D9980A4	79	38.5	25.3	61.8	79	39	25	62.4	Alive	79.5	39.6	25	62.5	Alive
Railroad	3DD.003D9980A8	59	28.3	15	18.7	59.15	28.5	15	19	Alive	59.1	28.4	16.4		Dead
Railroad	3DD.003D9980B0	67	32	17.2	29.5	67.4	32.4	17.4	29.7	Alive	67.6	31.6	18.3		Dead
Railroad	3DD.003D9980B8	79.15	38.5	22	53.6	79.6	40	22	52.6	Alive	80	39.4	22.3		Dead
Railroad	3DD.003D9980BB	78.3	37.3	20	38.9	78	36.4	19	36.9	Alive	78.6	37.4	19.7	39	Alive
Railroad	3DD.003D9980BC	78	37.25	21.4	45.4	78	39	22		Dead					Previously Dead
Railroad	3DD.003D9980BF	59	29.15	15.1	21.1					Missing					Missing
Railroad	3DD.003D9980C2	62.4	30	15.5	21.6					Missing					Missing
Railroad	3DD.003D9980C7	87.9	39.65	19.1	43.8	88.3	39	19	37.1	Alive	88.4	38.7	19.2		Dead
Railroad	3DD.003D9980CC	67.4	31	17	22.8	68	31.2	16	23.5	Alive	67.8	31.5	16.2		Dead
Railroad	3DD.003D9980D0	80.2	37.1	21.1	48.3	80	38.3	21.3		Dead					Previously Dead
Railroad	3DD.003D9980D3	87.45	41.2	22	60.3	88	41	22	59.1	Alive	88.2	39.7	23.3		Dead
Railroad	3DD.003D9980DE	73	35.3	19.6	40	73.5	35	20	39.4	Alive	73.8	35.1	21.3		Dead
Railroad	3DD.003D9980E0	82.7	37.2	21.5	47.7	82	36	22		Dead					Previously Dead

Table 3A. Fish species abundance found restoration reach of the Grand River, Michigan. Fish were collected using tote-barge or canoe-based electrofishing in the restoration reach.

Reach	Scientific Name	Common Name	Count	Size Range (cm)	Median Size (cm)
Blue Bridge to Pearl Street	Ambloplites rupestris	Rock Bass	40	5.0 - 23.5	12.5
	Ameiurus natalis	Yellow Bullhead	1	18.8	18.8
	Cyprinella spiloptera	Spotfin Shiner	25	4.0 - 9.0	7.0
	Etheostoma blennioides	Greenside Darter	3	6.0 - 7.6	6.6
	Ichthyomyzon castaneus	Chestnut Lamprey	1	20.0	20.0
	Lepomis cyanellus	Green Sunfish	10	6.0 - 11.8	8.2
	Lepomis gibbosus	Pumpkinseed	11	5.5 - 11.3	8.0
	Lepomis gulosus	Warmouth	1	9.0	9.0
	Lepomis macrochirus	Bluegill	54	4.5 - 15	7.9
	Lota lota	Burbot	1	19.5	19.5
	Micropterus dolomieu	Smallmouth Bass	6	10 - 32.5	18.8
	Micropterus salmoides	Largemouth Bass	10	4.6 - 24.8	13.2
	Neogobius melanostomus	Round Goby	34	5.5 - 15.0	9.5
	Notropis atherinoides	Emerald Shiner	18	4.0 - 6.5	5.7
	Notropis stramineus	Sand Shiner	45	3.0 - 9.5	4.7
	Notropis volucellus	Mimic Shiner	19	3.0 - 6.9	5.3
	Percina caprodes	Common Logperch	13	9.0 - 12.2	10.7
Gillette Bridge to Dam 3	Ambloplites rupestris	Rock Bass	22	5.5 - 20.0	13.7
	Cyprinella spiloptera	Spotfin Shiner	1	5.5	5.5
	Etheostoma blennioides	Greenside Darter	4	7.0 - 8.5	7.8
	Lepomis cyanellus	Green Sunfish	5	6.2 - 13.0	9.9
	Lepomis gibbosus	Pumpkinseed	8	7.5 - 21.5	12.0
	Lepomis gulosus	Warmouth	1	8.5	8.5
	Lepomis macrochirus	Bluegill	48	5.0 -14.8	8.6
	Lota lota	Burbot	1	22.0	22.0
	Micropterus dolomieu	Smallmouth Bass	1	32.0	32.0

	Micropterus salmoides	Largemouth Bass	4	14.0 - 23.0	20.3
	Neogobius melanostomus	Round Goby	22	7.0 - 13.5	9.5
	Notropis atherinoides	Emerald Shiner	30	3.5 - 8.0	6.0
	Notropis heterolepis	Blacknose Shiner	2	5.0 - 5.5	5.3
	Notropis stramineus	Sand Shiner	22	3.0 - 10.5	5.2
	Notropis volucellus	Mimic Shiner	12	4.0 - 7.0	5.0
	Percina caprodes	Common Logperch	7	9.0 - 12.1	11.0
Dam 3 to Bridge Street	Ambloplites rupestris	Rock Bass	27	5.5 - 20.5	13.1
	Ameiurus natalis	Yellow Bullhead	1	18.5	18.5
	Cyprinella spiloptera	Spotfin Shiner	16	5.2 - 10.5	7.9
	Etheostoma blennioides	Greenside Darter	13	6.5 - 8.5	7.7
	Etheostoma caeruleum	Rainbow Darter	2	5.2 - 6.5	5.9
	Hypentelium nigricans	Northern Hogsucker	1	12.8	12.8
	Lepomis cyanellus	Green Sunfish	1	6.5	6.5
	Lepomis gibbosus	Pumpkinseed	2	7.0 - 12.0	9.5
	Lepomis gulosus	Warmouth	1	10.2	10.2
	Lepomis gulosus X L. cyanellus	Warmouth X Green Sunfish	1	10.0	10.0
	Lepomis macrochirus	Bluegill	25	5.0 - 12.5	9.4
	Micropterus dolomieu	Smallmouth Bass	1	15.5	15.5
	Micropterus salmoides	Largemouth Bass	8	11.0 - 33.3	18.4
	Moxostoma erythrurum	Golden Redhorse	2	20.0 - 23.0	21.5
	Moxostoma macrolepidotum	Shorthead Redhorse	4	10.5 - 23.5	16.0
	Neogobius melanostomus	Round Goby	44	4.5 - 15.5	9.7
	Nocomis micropogon	River Chub	1	21.0	21.0
	Notropis atherinoides	Emerald Shiner	5	4.0 - 7.0	5.6
	Notropis heterolepis	Blacknose Shnier	7	4.0 - 6.0	4.6
	Notropis stramineus	Sand Shiner	36	3.0 - 7.5	4.5
	Notropis volucellus	Mimic Shiner	9	4.0 - 6.5	4.9
	Percina caprodes	Common Logperch	40	8.0 - 12.0	9.9
	Percina maculata	Blackside Darter	1	8.0	8.0
Canoe Surveys	Ambloplites rupestris	Rock Bass	4	9.0 - 15.6	15.3

Cyprinella spiloptera	Spotfin Shiner	3	6.0 - 10.5	8.4
Etheostoma blennioides	Greenside Darter	1	7.0	7.0
Lepisosteus osseus	Longnose Gar	1	71.0	71.0
Lepomis gibbosus	Pumpkinseed	4	9.0 - 15.6	13.1
Lepomis macrochirus	Bluegill	14	6.0 - 15.0	11.2
Micropterus dolomieu	Smallmouth Bass	1	22.5	22.5
Micropterus salmoides	Largemouth Bass	2	16.0 - 24.0	20.0
Moxostoma carinatum	River Redhorse	1	70.0	70.0
Moxostoma macrolepidotum	Shorthead Redhorse	11	15.0 - 40.5	30.4
Neogobius melanostomus	Round Goby	3	6.5 - 11.0	9.5
Notropis stramineus	Sand Shiner	10	4.0 - 6.5	5.0
Notropis volucellus	Mimic Shiner	14	4.5 - 7.0	5.5
Percina caprodes	Common Logperch	6	9.5 - 11.5	10.3

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