Survival, growth, and settlement of Dreissena rostriformis bugensis veligers in low and high calcium waters

Emma Ruhmann
University of Nevada, Las Vegas, emma.ruhmann@gmail.com
SURVIVAL, GROWTH, AND SETTLEMENT OF DREISSENA
ROSTRIFORMIS BUGENSI VELIGERS IN LOW
AND HIGH CALCIUM WATERS

By

Emma Kathleen Ruhmann

Bachelor of Science in Environmental Science
Saint Louis University
2010

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science - Water Resources Management

Department of Water Resources Management
College of Sciences
The Graduate College

University of Nevada, Las Vegas
August 2014
THE GRADUATE COLLEGE

We recommend the thesis prepared under our supervision by

Emma Kathleen Ruhmann

entitled

Survival, Growth, and Settlement of Dreissena Rostriformis Bugensis Veligers in Low and High Calcium Waters

is approved in partial fulfillment of the requirements for the degree of

Master of Science - Water Resource Management
Department of Water Resources Management

Michael Nicholl, Ph.D., Committee Chair
Craig Palmer, Ph.D., Committee Member
Kumud Acharya, Ph.D., Committee Member
Carl Reiber, Ph.D., Graduate College Representative
Kathryn Hausbeck Korgan, Ph.D., Interim Dean of the Graduate College

August 2014
ABSTRACT

SURVIVAL, GROWTH, AND SETTLEMENT OF DREISSENA ROSTRIFORMIS BUGENSI VELIGERS IN LOW AND HIGH CALCIUM WATERS

By

Emma Kathleen Ruhmann

Michael Nicholl, Ph.D., Examination Committee Chair
Kumud Acharya, Ph.D., Examination Committee Co-Chair
Water Resources Management Program
University of Nevada, Las Vegas

Populations of *Dreissena rostriformis bugensis* (quagga mussels) have continued to spread throughout the western United States since their discovery in the Boulder Basin of Lake Mead, NV-AZ in early 2007. Today, quagga mussel specific research is still lacking and the physicochemical characteristics of aquatic systems required by quagga mussels to successfully establish is not fully understood. This includes an absence of research in aquatic environments in the western United States and on quagga mussel veligers (larval stage). Calcium is considered the defining factor for determining if a lake or river is suitable for quagga mussel establishment. The minimum calcium threshold for invasion was developed in prior studies using the calcium requirements of zebra mussels, a close relative to the quagga mussels. Research has shown that there are many differences between the two species and the risk of quagga mussel survival in low calcium waters might be underestimated. This study sought to fill the gaps in quagga...
mussel veliger research. Three parameters (survival, growth, and settlement) were used to
determine their potential effectiveness of establishment in aquatic environments with
varying levels of dissolved calcium. To study the potential of veligers to survive, grow,
and settle, three bioassays were completed. The first analyzed survival and growth of
veligers in two different systems (Lake Mead and Lake Tahoe). Lake Tahoe, CA-NV was
chosen as the system to represent naturally low calcium levels (approximately 12 ppm
Ca). Quagga mussels have yet to establish in the Lake. To represent a system of high
calcium, water from Lake Mead, NV-AZ (approximately 70 ppm Ca) was used. This
assay concluded that while veligers raised in the Lake Tahoe water had a lower survival
rate than those in the Lake Mead water, those veligers which survived grew at an equal
growth rate. The second assay looked at survival and growth with three additional water
treatments (Lake Tahoe with Ca amended to approximately 20, 25, and 32 ppm). The
added calcium in the Lake Tahoe water helped to increase survival; and again, growth
rates among all five treatments were very similar. Both survival and growth assays
showed that those veligers which survived the 28 day assay grew at a similar rate no
matter the calcium level of the treatment water. The third assay was designed to
determine the settlement potential of veligers raised in three different treatment waters
(Lake Mead, Lake Tahoe, and Lake Tahoe with 25 ppm Ca). Veliger settlement is an
important life event because it indicates that the veligers have found a suitable habitat to
remain in and metamorphose into the adult stage. Results indicate that the percent of
settlement was improved with increasing levels of calcium. The findings from these
assays will aid in aquatic invasive species management. They were designed so that they
can be replicated using water from other western lakes with low levels of calcium to test
the potential for quagga mussel establishment. Quagga mussels cause both economic and ecological impacts. It is important to better understand them in hopes of preventing their spread to other systems and minimizing their impact on the environment.
A sincere Thank You to my Advisor, Dr. Kumud Acharya: for your guidance and support. Thank you for providing me with all the resources necessary to complete this Thesis.

Thank You to my Committee Chair and Members: Dr. Michael Nicholl, Dr. Craig Palmer, and Dr. Carl Reiber: for taking time out of your busy schedules to support my endeavors.

Thank You to my partners in crime, Dr. Sudeep Chandra and Dr. Clinton Davis at the University of Nevada, Reno: for developing and making this project a reality.

Finally to Kristina Hsu, Sachiko Sueki, and Elle Law: We counted and documented thousands of veligers in just a few short months. It was not only impressive but a fun and enjoyable experience. Thank You for our adventures.
Dedicated to my mother and father:

For providing me with the resources and guidance needed

to become an educated and empowered young woman striving to make a
mark on society.
# Table of Contents

Abstract .......................................................................................................................... iii

Acknowledgements ......................................................................................................... vi

Dedication ....................................................................................................................... vii

List of Tables .................................................................................................................. xi

List of Figures .................................................................................................................. xii

Chapter 1 - Introduction .................................................................................................. 1

1.1 - Background on Quagga Mussels .......................................................................... 1

1.2 - Calcium as a Colonizing Control ........................................................................... 4

1.3 - An Introduction to the Survival, Growth, and Settlement Assays ......................... 7

1.4 - Significance of Research and Results ................................................................... 9

1.4.1 - Quagga Mussels Research ............................................................................... 9

1.4.2 - Ecological Impact ............................................................................................ 11

1.4.3 - Economic Impact .............................................................................................. 12

1.4.4 - Future Lake Tahoe Management .................................................................... 13

Chapter 2 - Lake Mead versus Lake Tahoe Comparative Survival and Growth Assay ... 15

2.1 - Introduction ........................................................................................................... 15

2.2 - Methods ................................................................................................................ 16

2.2.1 - Collection, Preparation, and Water Analysis of Culture Water ....................... 17

2.2.2 - Veliger Collection and Identification ................................................................ 21
Appendix 2. Statistical Analysis ................................................................. 60

Appendix 3. Brochure Used to Educated Boaters and the Public on Boat Inspections and the Spread of Invasive Species in Nevada ................................................................. 61

Appendix 4. Nevada Department of Wildlife Special Collection Permit .................. 63

Bibliography ................................................................................................. 68

Vita................................................................................................................. 76
List of Tables

Table 1.1. Invasion risk classifications for quagga and zebra mussels based on calcium concentration................................................................. 5

Table 1.2. Aquatic systems in the Colorado River Region where Dreissenids have been detected despite lower dissolved calcium levels................................................................. 7

Table 2.1. Summary of water quality results from samples collected at Lake Mead and Lake Tahoe........................................................................... 20

Table 2.2. Veliger survival and growth rate comparing Lake Tahoe and Lake Mead treatments .......................................................................... 29

Table 3.1. Calcium (Ca) concentration of treatment waters................................................................................................................. 38

Table 3.2. Veliger survival and growth rate comparing treatments of varying calcium levels after 28 Days................................................................................. 40

Table 4.1. Calcium (Ca) concentration of treatment waters................................................................................................................. 51

Table 4.2. Number of total veligers settled after 30 Days comparing low, high, and elevated calcium treatments......................................................... 53
List of Figures

Figure 1.1. The approximated potential range limits for zebra mussels in North America as developed by Strayer, 1991 based on the range of temperature tolerated by the species in Europe.........................3

Figure 1.2. Current location map of aquatic systems where zebra and quagga mussels have established .................................................................4

Figure 1.3. Dreissenid invasion risk classes for the contiguous United States based on calcium concentrations in the aquatic systems .....................................6

Figure 1.4. Brochure used to educate boaters and the public on boat inspections in the western states indicating ways to prevent the spread of invasive species and a list of contacts by state ........................................................................11

Figure 1.5. Brochure used to educated boaters and the public on boat inspections at Lake Tahoe indicating ways to prevent the spread of invasive species.........................14

Figure 2.1. Map of Lake Mead, NV-AZ.........................................................18

Figure 2.2. Map of Lake Tahoe, CA-NV........................................................19

Figure 2.3. Image quagga mussel veliger under cross polarizing light showing the characteristics "iron cross" used to identify the species.. .................................22

Figure 2.4. Veliger displaying behavior of cilia movement on an extended velum. .......24

Figure 2.5. Veliger displaying behavior of foot extended and moving ....................25

Figure 2.6. Veliger displaying behavior of internal organs moving indicating digestion 25

Figure 2.7. Veliger displaying behavior of swimming with the velum fully extended. ...26

Figure 2.8. Deceased veliger displaying an open shell and decomposed internal organs 26
Figure 2.9. Plantigrade life stage displaying the elongated shape and thickening of the shell. ................................................................. 27
Figure 2.10. Determining shell area of surviving veligers using Image J. ............... 28
Figure 2.11. Average rate of survival comparing Lake Tahoe versus Lake Mead treatments ........................................................................................................................................ 30
Figure 2.12. Average area of shells for surviving veligers comparing Lake Tahoe versus Lake Mead treatments ........................................................................................................................................ 31

Figure 3.1. Lake Tahoe water with additional calcium aerated and stored in laboratory. 39
Figure 3.2. Average rate of survival comparing low, high, and amended calcium treatments ........................................................................................................................................ 40
Figure 3.3. Average area of shells for surviving veligers comparing low, high, and calcium amended treatments ........................................................................................................................................ 42

Figure 4.1. Veligers settling in Imhoff cones at ambient laboratory temperature for two hours ........................................................................................................................................ 49
Figure 4.2. Glass tiles lining the bottom of the settlement tanks showing the numbering of the tiles ........................................................................................................................................ 52
Figure 4.3. Settlement tanks in separate secondary containment (per laboratory containment protocols) with lighting and aeration. ........................................................................................................................................ 52
Figure 4.4. Veligers attached to a glass tile during settlement assay. Mussels are shown using siphons to filter water ........................................................................................................................................ 54
CHAPTER 1 - INTRODUCTION

1.1 - Background on Quagga Mussels

*Dreissena rostriformis bugensis* (quagga mussels) is a bivalve species native to the Dnieper River in Eastern Europe (Mills et al., 2006). The quagga mussel is related to another invasive species, the more widely studied *Dreissena polymorpha*, (zebra mussels). Quagga mussels were first identified in North America in Lake Ontario (Great Lakes region) in 1992 (Nichols and Black, 1994). It is believed that both quagga and zebra mussels were originally transported to North America in the ballast water of ships (Van der Velde et al., 2010).

In the Great Lakes, quagga mussels originally preferred deeper waters and their initial expansion was slow. Comparatively, zebra mussels preferred shallower waters and rapidly expanded throughout the Great Lakes, (Whittier el al., 2008). More recently, quagga mussels spread into areas previously dominated by zebra mussels and colonized additional aquatic systems. Within the Great Lakes and St. Lawrence River, they began to expand to shallow water (Stoeckmann, 2003; Jones and Ricciardi, 2005). In January 2007, quagga mussels were discovered in the Boulder Basin of Lake Mead, NV-AZ (100th Meridian, 2011). Since then, they have continued to spread in the western United States, including: California, Nevada, Arizona, Utah, and Colorado (Hickey, 2010; 100th Meridian, 2011). In particular, the Lower Colorado River system (Nevada, California, and Arizona) hosts large densities of quagga mussels (100th Meridian, 2011).

Dreissenid species can invade an aquatic system through both anthropogenic and natural (also known as secondary) invasion mechanisms. The adult mussels attach to the
hard surfaces of boats and marine equipment (e.g., trailers, cranes, etc.) and can thus be transported upstream or overland. The free floating larval stage (veligers) can be transported in marine equipment that retains water (e.g., boat bilge water, bait and live wells, etc.). Anthropogenic transportation is likely the cause of quagga mussels spreading from the Great Lakes to Lake Mead (Hickey, 2010). Secondary invasion takes place when a species moves through interconnected waterways, establishing in small streams, rivers, and inland lakes. The free floating veligers move naturally with water currents, with a preference for downstream transport. The spread of the mussels throughout the Colorado River Basin was likely a combination of anthropogenic and natural transport mechanisms.

Prediction of the future spread of quagga and zebra mussels has focused on aquatic conditions, with limited research specific to quagga mussels. Strayer (1991) developed a model predicting zebra mussel future expansion in large, calcium rich lakes and rivers based on the range of temperatures tolerated in Europe and Russia (Figure 1.1). Subsequent spread of zebra mussels south of Strayer’s predicted southernmost limits (Figure 1.2) called into question the concept of water temperature as a limiting factor. However, other studies have supported the theory that dissolved calcium concentration places a control on colonization by zebra and quagga mussels (Hincks and Mackie, 1997; Cohen and Weinstein, 2001; Jones and Ricciardi, 2005).
Figure 1.1. The approximated potential range limits for zebra mussels in North America as developed by Strayer, 1991 based on the range of temperature tolerated by the species in Europe. The solid lines represent mean annual air temperatures of 0-18 °C. The dotted lines represent the lowest monthly mean air temperature of -15 °C and highest monthly mean air temperature of 27 °C. (from Strayer, 1991).
Figure 1.2. Current location map of aquatic systems where zebra and quagga mussels have established. Compared to Figure 1.1, both species have established in systems south of Strayer's predictions (from USGS Nonindigenous Aquatic Species Database, 2014).

1.2 - Calcium as a Colonizing Control

Dreissenid species, including quagga and zebra mussels, extract calcium from the environment to use for shell construction, growth, and physiological functions (Baldwin et al., 2012). The general consensus is that zebra mussels are unable to colonize in systems with low concentrations (<8-12 ppm) of calcium (Neary and Leach, 1992; Ramcharan et al., 1992; Mellina and Rasmussen, 1994; McMahon, 1996; Hincks and Mackie, 1997; Frischer et al., 2005; Jones and Ricciardi, 2005; Whittier et al., 2008). As illustrated in Table 1.1 and Figure 1.3, calcium concentration has been proposed as a
metric to assess the risk of quagga and zebra mussel invasion in the United States (Whittier et al., 2008). This assessment was based on published studies for zebra mussels, and assumed that calcium requirements for quagga mussels would be similar.

Initial calcium studies suggested a similar range in calcium requirements for quagga and zebra mussels (Whittier et al., 2008). However, significant differences between the two species have been found with respect to: environmental tolerances, bioenergetics, growth, shell mass, and colonization patterns (Mills et al., 1996; Claxton et al., 1998; Mills et al., 1999, Baldwin et al., 2002; Stoeckmann, 2003; Casper and Johnson, 2010). Therefore, it is important to make a species-specific assessment of calcium needs for quagga mussels.

Table 1.1. Invasion risk classifications for quagga and zebra mussels based on calcium concentration (from Whittier et al., 2008).

<table>
<thead>
<tr>
<th>Risk class</th>
<th>Distribution of calcium concentrations at sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low</td>
<td>75th percentile &lt; 12 mg L&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low</td>
<td>12 mg L&lt;sup&gt;-1&lt;/sup&gt; ≤ 75th percentile &lt; 20 mg L&lt;sup&gt;-1&lt;/sup&gt; or 75th percentile &lt; 21 mg L&lt;sup&gt;-1&lt;/sup&gt; and maximum &lt; 28 mg L&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>High</td>
<td>mean ≥ 28 mg L&lt;sup&gt;-1&lt;/sup&gt; and 25th percentile &gt; 12 mg L&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Highly variable</td>
<td>≥ 15% of sites with Ca &lt; 12 mg L&lt;sup&gt;-1&lt;/sup&gt; AND ≥ 15% of sites with Ca ≥ 28 mg L&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Figure 1.3. Dreissenid invasion risk classes for the contiguous United States based on calcium concentrations in the aquatic systems (from Whittier et al., 2008).

In the western United States, invasive dreissenids have been found in aquatic systems with lower and more varied calcium concentrations than previously reported (Chandra et. al., 2009). Quagga and/or zebra mussels have successfully established in eight Coloradan water systems (Table 1.2). Four of those systems (Grand Lake, Shadow Mountain, Willow Creek Reservoir, and Lake Granby) have calcium levels (3.5 to 19.1 ppm calcium) that qualify for the very low or low risk categories in the classification scheme proposed by Whittier et al. (2008).
Table 1.2. Aquatic systems in the Colorado River Region where Dreissenids have been detected despite lower dissolved calcium levels (from Chandra et al, 2009).

<table>
<thead>
<tr>
<th>Ecosystem</th>
<th>Dreissenid type</th>
<th>Life stage</th>
<th>Calcium (mg L⁻¹)</th>
<th>Chlorophyll a (μg L⁻¹)</th>
<th>Total P (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grand Lake</td>
<td>quagga, zebra</td>
<td>veliger</td>
<td>4.0-9.0</td>
<td>0.8-7.1</td>
<td>0.006-0.032</td>
</tr>
<tr>
<td>Shadow Mtn Res.</td>
<td>quagga</td>
<td>veliger</td>
<td>3.5-9.5</td>
<td>0.5-25.2</td>
<td>0.009-0.030</td>
</tr>
<tr>
<td>Willow Creek Res.</td>
<td>quagga</td>
<td>veliger</td>
<td>10.4-19.1</td>
<td>5.6-8.3</td>
<td>0.009-0.012</td>
</tr>
<tr>
<td>Lake Granby</td>
<td>quagga</td>
<td>veliger</td>
<td>5.5-11.0</td>
<td>1.0-12.8</td>
<td>0.006-0.038</td>
</tr>
<tr>
<td>Pueblo Res.</td>
<td>quagga, zebra</td>
<td>veliger</td>
<td>23.75</td>
<td>4.5-51.8</td>
<td>0.005-0.097</td>
</tr>
<tr>
<td>Jumbo Res.</td>
<td>quagga</td>
<td>veliger</td>
<td>26-47</td>
<td>0.5-102</td>
<td>&lt;0.01-0.14</td>
</tr>
<tr>
<td>Tarryall Res.</td>
<td>quagga</td>
<td>veliger</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Blue Mesa Res.</td>
<td>quagga</td>
<td>veliger</td>
<td>27.5-37.9</td>
<td>N/A</td>
<td>0.008-0.057</td>
</tr>
</tbody>
</table>

1.3 - An Introduction to the Survival, Growth, and Settlement Assays

Successful colonization requires that quagga mussel veligers be able to survive, grow, and settle in their new environment. This thesis presents laboratory experiments designed to analyze the potential of quagga mussel veligers to survive, grow, and settle in natural waters from the western United States that exhibit varying calcium concentrations. Water used in the experiments was taken from either Lake Mead (high calcium) or Lake Tahoe (low calcium).

Lake Mead, NV-AZ was chosen as a control due to its high calcium levels and the already successful establishment of quagga mussels in the Lake. Calcium levels measured in Lake Mead outflow in 2001-2009 indicated an average calcium level of 76.0 ppm (Holdren and Turner, 2010) placing the Lake in the ‘high risk’ category as defined by Whittier et al. (2008).
Lake Tahoe, CA-NV was chosen due to its low levels of calcium. Lake Tahoe is one of the large, western aquatic systems that has so far evaded colonization by quagga mussels (Figure 1.2). The calcium assessment ranked Lake Tahoe as a ‘low risk’ lake for quagga mussel establishment (Whittier et al., 2008) based on the Lake’s low (6 to 14 ppm, Chandra et al., 2009) dissolved calcium concentrations. As an alpine lake, Lake Tahoe averages a surface water temperature ranging from 4.5 to 21°C (Tahoe USGS). This is in comparison to the surface water temperature at Lake Ontario in the Great Lakes (where quagga and zebra mussels have successfully established) that ranges from 2.4 to 22.5°C (NOAA).

Lake Tahoe has not been immune to other invasive species establishment. A similar species, *Corbicula fluminea* (Asian clams), has already established in the Lake. A small number of Asian clams were found in Lake Tahoe in 2002 with high density populations (up to 6000 clams m$^{-2}$) observed in 2008 (Wittmann et. al., 2012). In spring 2008, Asian clams were found in extensive and dense beds in the southeastern Lake Tahoe areas from Zephyr Cove to El Dorado Beach. The clams contribute calcium to the system through the degradation of their shells causing regions of elevated calcium levels. These elevated regions may facilitate the invasion of quagga mussels (State of the Lake Report, 2009).

The subsequent Chapters present experiments conducted to answer the following questions. First, Chapter 2 will compare veliger survival and growth in Lake Mead and Lake Tahoe to determine first if veligers are able to survive and grow in Lake Tahoe and secondly to compare their growth rate in the two Lakes. Second, Chapter 3 will further investigate the veliger’s potential to survive and grow in Lake Tahoe by using treatments
of Lake Tahoe water with calcium additions. Third, Chapter 4 will address the ability of veligers to settle comparing the rate of settlement between the two Lakes. Finally, in conclusion, Chapter 5 will summarize the results from the assays conducted and provide suggestions for future work on this topic.

1.4 - Significance of Research and Results

The studies presented in the following Chapters will help the management of quagga mussels, work to prevent their continual spreading, and answer some questions of the environmental requirements of quagga mussel veligers. Specifically, experiments will study the ability of veligers to live in low calcium water and determine what levels they prefer. It will also provide information on quagga mussels specific to waters in the western United States; growth, survival, and settlement studies on veligers have until now not been conducted in western waters. It will help provide viable information to lake and river managers in the western United States who are concerned with the invasive establishment of quagga mussels. Policy and decision makers in water management rely on scientific research to ensure the best preventive management is implemented and that financial support is appropriately allocated and spent. Finally, these studies provide a foundation for additional studies of quagga mussel invasion in the western United States. These experiments may be replicated using water from other lakes and rivers of concern for invasion.

1.4.1 - Quagga Mussels Research

To date, quagga mussel research is lacking in many key areas of study. First, the calcium risk assessment was developed using zebra mussels and may not accurately
define the calcium requirements of quagga mussels. Second, survival, growth, and settlement laboratory experiments using veligers have not been conducted using waters specific to the western United States. Thirdly, general research specific to quagga mussels has remained in limited presence in scientific journals.

Due to the currently low amount of quagga mussel specific research (especially in the western United States), this study adds to the general knowledge of quagga mussel veligers. We stretched the study to its lengths using the longest possible period for the experimental assays. This exceeded previous veliger studies under laboratory conditions. The study also focused on two important Lakes in the western United States comparing their conditions necessary for successful veliger establishment. The data provided builds on the understanding of the importance of calcium to quagga mussel veligers. It can be applied to other low calcium lakes in the western United States (Figure 1.4).
Figure 1.4. Brochure used to educate boaters and the public on boat inspections in the western states indicating ways to prevent the spread of invasive species and a list of contacts by state (Pamphlet received in Person).

1.4.2 - Ecological Impact

Invasive species have an impact on the ecology of the area in which they establish. Quagga mussels especially are considered to have a significant ecological impact. The mussels filter the water removing large amounts of phytoplankton. This reduces the availability of prey for the higher tropic levels (Hickey, 2010). They dominate and alter lake and stream ecology impacting all trophic levels, especially benthic invertebrate communities (Wittmann et al. 2010). This may in turn decrease algal biomass and alter nutrient availability (Chandra et al. 2009).
As mussels filter organic material from the water, they release ammonium in their pseudofeces, producing some of the highest nitrogen excretion rates of any animal (Bruesewitz, 2006). As the pseudofeces decompose, oxygen is consumed causing the surrounding water to become more acidic (Hickey, 2010). Quagga mussels are also known to have levels of organic pollutants over 300,000 times greater than environmental concentrations in their tissues (Snyder et al., 1997)

Zebra mussels can act as a phosphorus sink in phosphorus-limiting environments (Johengen et al. 1995). They have an effect on water column phosphorus when the phosphorus output of the mussel is greater than 20% of the lake’s phosphorus (Mellina et al. 1995).

### 1.4.3 - Economic Impact

Quagga and zebra mussels have caused large amounts of damage to infrastructure in lakes and rivers. They clog water intake pipes, water filtration, and electric generating plants (Pimentel et al., 2005). The mussels not only attached to these structures but clumps of empty shells also become dislodged and trapped inside the equipment. It is estimated that quagga and zebra mussels cause $1 billion dollars in damages and associated control costs every year (Pimentel et al., 2005). Between the introduction of quagga mussels to Lake Mead in January 2007 and 2009, the Southern Nevada Water Authority (SNWA) spent an estimated $32 million managing the impacts of the quagga mussels on the water intake infrastructure at the Lake (Roefer, P., 2009).

Millions of dollars have been spent to remove the mussels from dam infrastructure, including costly removal at Hoover Dam, NV-AZ (BOR, 2009). Due to their effect on ecology, their invasion in Lake Huron has caused populations of salmon,
alewife, zooplankton, and phytoplankton to drastically decline causing a $19 million dollar per year drop in revenue for sport fishing in the Lake (Michigan DNR, 2008).

### 1.4.4 - Future Lake Tahoe Management

A pilot study was conducted in 2009 to determine the possibility of successful quagga mussel establishment in Lake Tahoe, should the species be introduced. The study found evidence of successful adult survival but asked for additional information on the quagga mussel veligers specifically. The results presented here fill in the gaps left by the pilot study to understand the ability of quagga mussel veligers to survive, grow, and settle in Lake Tahoe. The Tahoe Region depends on research like this to continue and add to the measures in place for preventing aquatic invaders.

Currently, boats are inspected and washed prior to entering Lake Tahoe during the boating season (Figure 1.5). Findings from this study will help future decisions on the investments of the costly inspection activities.
Figure 1.5. Brochure used to educated boaters and the public on boat inspections at Lake Tahoe indicating ways to prevent the spread of invasive species (Pamphlet received in person from Invasive Species Program, Lake Tahoe EIP).
2.1 - Introduction

Survival and growth rates are two of the defining parameters to determine the success of invasive establishment. To test the ability of quagga mussel veligers to establish in Lake Tahoe, the following experiment was conducted by raising veligers in a semi-natural laboratory setting in two different treatments. Veligers collected from Lake Mead were maintained in both water from Lake Mead (acting as a control) and Lake Tahoe. The survival and growth rates of the veligers were compared between the two treatments. The assay simulates the movement of veligers from one ecosystem to another (Lake Mead to Lake Tahoe) as if they were transferred between lakes by anthropogenic means. Results from this study will help with management and prevention of quagga mussels in other low calcium systems in the western United States.

Previous experimental studies have attempted to better understand the use of calcium by quagga mussels and how calcium limits their invasive spread. Two studies of note used waters of varying calcium levels from systems in the eastern United States to maintain quagga mussels in a laboratory setting and determine their survival and growth (Jones and Ricciardi, 2005 and Baldwin et al., 2012). This assay presented is unique in that it studies the calcium impact of quagga mussel veligers in waters in the western United States. Baldwin et al. (2012) was the first to publish survival and growth assays specific to quagga mussels. They were less confident in the ability of veligers to invade some regions in New York State than we are of their ability to establish in some of the lower calcium environments in the western United States.
In this study, growth rate is one of the defining parameters to determine the success of invasive establishment. Surviving veliger shell area was measured during the experiment and a growth rate per day was determined. Growth rate is the rate at which an organism increases in size per unit of time and determines the probability of survival and fecundity (Arendt, 1997). Positive growth rate of the veligers will indicate that the environmental conditions are suitable to their needs. Quagga mussel veligers need to grow quickly in order to avoid predation and reach maturity.

**Questions**

Can veliger quagga mussels survive and grow in water from Lake Tahoe? How does their survival and growth rate compare to that of veligers maintained in water from Lake Mead?

**Hypothesis**

Veliger quagga mussels raised in Lake Tahoe water will exhibit a lower growth rate and survivorship potential than those raised in Lake Mead water due to the lower levels of dissolved calcium in the Lake Tahoe environment.

**2.2 - Methods**

Long term veliger culturing in a laboratory setting is difficult to achieve, based on previous studies. The previous survival and growth study with the longest observation period was 10-14 days (Baldwin et al., 2012). A maximum laboratory growth period of 28 days was achieved for these experimental assays. For the six months prior to the start of this assay, trial experiments were completed to determine the ideal culturing conditions. Based on this laboratory experience, it was determined that veliger health
(regardless of water type) begins to deteriorate after four to six weeks in a laboratory setting. Survival and growth assays were limited to 28 days to lessen the negative effects of laboratory growth while maximizing the number of days where measurement photographs could be taken.

2.2.1 - Collection, Preparation, and Water Analysis of Culture Water

Water for all assays was collected from Lake Mead and Lake Tahoe on a regular basis. Lake Mead water was collected from the docks at the Callville Bay Marina (36°01’29.51” N, 114°46’21.06” W) and transported in (25 and 10 L) Nalgene® carboys (Figure 2.1). Callville Bar Marina was selected due to the long docks which sit low in the water and helped ease water collection. Carboys were triple rinsed with Lake water prior to collection. Lake Tahoe water was collected from the Tahoe Keys boat launch (38°55’45.98”N, 120°00’51.84”W) using a 400 gallon water wagon (rinsed prior to collection) and delivered to Las Vegas as needed (Figure 2.2). The Tahoe Keys area was chosen because the high volume of boater activity makes it a likely site for introduction and the average dissolved calcium concentration is higher than in other parts of the lake (Chandra et al., 2009). Once in the lab, all water was filtered through a 35 µm Nitex mesh filter held between a PVC ring cap and bushing of 2 inch diameter (Schwaebel et al., 2013) to remove veligers, competing zooplankton, and any foreign bodies from the water. Both Filtered Lake Mead Water (FLMW) and Filtered Lake Tahoe Water (FLTW) was stored in covered 20 L plastic, opaque buckets with aeration and kept at ambient laboratory temperature (approximately 20 °C).
Figure 2.1. Top: Map of Lake Mead, NV-AZ. Red square highlighting the Boulder Basin and red star highlighting the Callville Bay Marina. Below: Callville Bay Marina with circle highlighting the collection area (adapted from Google Maps, 2014a).
Figure 2.2. Top: Map of Lake Tahoe, CA-NV. Red star highlighting the Tahoe Keys. Below: Tahoe Keys with red circle highlighting the collection area (adapted from Google Maps – Tahoe, 2014b).
Chemical analysis of the culture water is shown as Table 2.1. Discrete water quality parameters (dissolved oxygen and temperature) were measured on site during collections. Post-filtration samples of FLMW and FLTW were analyzed by a third party water analysis laboratory for nutrient constituents (ammonia, nitrate, calcium, magnesium, potassium, and sodium) as well as algal biomass (chlorophyll a). See Table 2.1 for a summary of the results. Lake Tahoe is defined as an oligotrophic system and therefore has lower nutrient levels compared to the water from Lake Mead (North Lake Tahoe, 2011).

**Table 2.1.** Summary of water quality results from samples collected at Lake Mead and Lake Tahoe. Numbers represent an average of samples taken during the course of all three experiments. * Data unavailable due to faulty probes. ** Chlorophyll a data may not be representative of the levels in the Lakes due to the location in the Lakes in which the data was collected. FLMW data was collected in the middle of Lake Mead while FLTW data was collected at a heavily populated marina.

<table>
<thead>
<tr>
<th></th>
<th>NH(^3)</th>
<th>NO(^-3)</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>K</th>
<th>DO</th>
<th>Chlor a**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg L(^{-1}))</td>
<td>(µg L(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLMW</td>
<td>0.013</td>
<td>0.437</td>
<td>76.0</td>
<td>21.50</td>
<td>75.85</td>
<td>4.01</td>
<td>n/a*</td>
<td>1.68</td>
</tr>
<tr>
<td>FLTW</td>
<td>0.002</td>
<td>0.014</td>
<td>12.0</td>
<td>2.42</td>
<td>7.02</td>
<td>1.43</td>
<td>9.88</td>
<td>19.8</td>
</tr>
</tbody>
</table>

As waste water was removed during the assays, ammonia (using Hach Water Quality Test Strips for Ammonia) and pH (using pHydron Insta-Chek Test Strips) was tested to ensure a stable environment remained in each petri dish. Ammonia was tested for anything over 1ppm and pH was tested for anything outside the natural range of the Lake Mead or Lake Tahoe systems of 7 to 8. The average pH of the outflow water at Lake Mead from 2001 to 2009 was 8.01 (Holdren and Turner, 2010). The pH of the Lake Tahoe sample water taken in the lab ranged from 7.5 to 8.06.
2.2.2 - Veliger Collection and Identification

All veligers used in this study were collected from along the docks at the Callville Bay Marina at Lake Mead using a 63 µm plankton net. The plankton net was sunk to approximately 12 meters below the water surface and towed horizontally. For this assay, veligers were collected in early May with an average water temperature at Callville Bay of 20 ºC (Resorts, 2014). During the spring months, the contents of the tows were filtered (250 µm) to remove large predators and pollen (Schwaebbe, 2012). Fewer predators were observed during the summer months and samples were returned to the laboratory unfiltered. Samples were transferred into plastic sampling bottles and transported to the laboratory in chilled coolers. Once in the laboratory, samples were combined into large plastic beakers and allowed to settle for one hour at ambient laboratory temperature (approximately 20 ºC) prior to identification and selection of experimental specimens.

Organisms selected for experiments were active quagga mussel veligers in the umbonal stage with a shell area of ~0.037 mm². A 1.5 mL transfer pipette was used to transfer samples of water from the bottom of the beakers onto a watch glass for observation. Organisms were identified as quagga mussel veligers using cross polarized light and a Nikon SMZ1000 microscope at 10x magnification. Quagga mussel veligers are distinguished from other species by the characteristic “iron cross” (Figure 2.3) on their shell when observed under cross polarized light (Johnson, 1995). Large (shell area of ~0.037 mm²) and active veligers (behaviors of cilia movement on an extended velum, foot extension, internal organ movement, swimming, and death are displayed in Figures 2.4–2.8) in the umbonal life stage (Figure 2.3) were hand selected, individually pipetted from the watch glass, and transferred to a petri dish. The unbonal stage is easily identified
as the hinge line grows from straight (in the previous straight-hinged stage) to curve outwards as the umbo become distinct (Nichols and Black, 1994). The shell remains transparent at this stage allowing for visual observations of digestion and organ health.

Petri dishes were filled with 45 mL of either FLMW or FLTW in replicates of five (25 veligers per treatment). Water (20 mL) was removed from the petri dish and replaced with fresh water twice per week. Petri dishes were kept in a laboratory incubator (VWR International Model #2015) at 19 ºC with light timers to ensure 8 hours of fluorescent incubator lighting daily for the course of the assay.

![Image quagga mussel veliger under cross polarizing light showing the characteristics “iron cross” used to identify the species. Arrow highlights the umbo. (Nikon SMZ1000 stereomicroscope at 20x magnification).](image_url)

**Figure 2.3.** Image quagga mussel veliger under cross polarizing light showing the characteristics “iron cross” used to identify the species. Arrow highlights the umbo. (Nikon SMZ1000 stereomicroscope at 20x magnification).
2.2.3 - Behavioral Observations and Life Stages

Veligers were observed around 1:00 PM on selected days during the course of the 28 day assays to observe behavior and assess mortality. Recorded behaviors include: cilia movement on an extended velum (Figure 2.4), foot extension (Figure 2.5), internal organ movement indicating digestion (Figure 2.6), swimming (Figure 2.7), and death (Figure 2.8) were recorded. The velum is a “ciliated swimming organelle” (Claudi and Mackie, 1994) which is used for both swimming and feeding. The foot of the veliger is an “extensible muscular midventral organ” used for locomotion (Nichols and Black, 1994). The four living behaviors are considered to represent a healthy and active veliger during the umbo and the succeeding life stages.

To determine the survival percentage of veligers in the assays, mortality was recorded during behavior observations. A veliger was considered deceased when the shell was open and either empty or organs showed visual signs of decomposition (Figure 2.8). Deceased veligers were left in the petri dish for the remainder of the assay. Ammonia levels were measured in waste water from the petri dishes twice weekly to monitor any changes to the water chemistry that might have occurred due to decay.

In addition to observing behaviors as indicators of health and growth, veligers were observed to detect the metamorphism from the initial umbo stage used at the start of the assays to the succeeding pediveliger and then plantigrade stages. To add in the observation, distinct characteristics to each stage were used to distinguish transformations. During the pediveliger stage, the shell begins to thicken and become more opaque. When the veliger reaches the plantigrade stage (Figure 2.9), the velum is reabsorbed and the veliger relies on the foot as their main means of locomotion (Nichols
and Black, 1994). Also during the plantigrade stage, the growth plates in the shell reorient. This causes the shell to transform from the round, clam shape into the elongated shape of the mussel (Nichols and Black, 1994).

Figure 2.4. Veliger displaying behavior of cilia movement on an extended velum (Nikon Eclipse TS100 inverted routine microscope at 200x magnification).
Figure 2.5. Veliger displaying behavior of foot extended and moving (Nikon SMZ1000 stereomicroscope at 80x magnification).

Figure 2.6. Veliger displaying behavior of internal organs moving indicating digestion (Nikon Eclipse TS100 inverted routine microscope at 200x magnification).
Figure 2.7. Veliger displaying behavior of swimming with the velum fully extended (Nikon SMZ1000 stereomicroscope at 80x magnification).

Figure 2.8. Deceased veliger displaying an open shell and decomposed internal organs (Nikon Eclipse TS100 inverted routine microscope at 200x magnification).
Figure 2.9. Plantigrade life stage displaying the elongated shape and thickening of the shell (Nikon SMZ1000 stereomicroscope at 80x magnification).

2.2.4 - Digital Imagery Analysis and Growth Rate Measurement

Digital photographs of each individual veliger were taken on Days 0, 7, 14, 21, and 28 using an Infinity 2 camera attached to the Nikon SMZ1000 microscope at 80x magnification. The advantage of estimating growth by digital image analysis is that it is a minimally invasive method. The growth rate can be measured on an individual basis while keeping the animal alive for subsequent growth (Acharya et. al., 2004). At the conclusion of 28 days, all pictures of living veligers were measured, using Image J (1.47v Java 1.6.0_20, National Institutes of Health, USA), for shell area (Figure 2.10) following the procedure developed by Acharya et al. (2004). Image J displayed shell area to three decimal places. The change in shell area after 28 days was used to determine rate of
growth (also calculated to the same significant figures) using Equation 2.1 (adapted from Acharya et al., 2004). Results were analyzed as a comparison across the water treatments.

$$\mu = \frac{\ln (A_2/A_1)}{t}$$

Eq. 2.1

Where:

$$\mu = \text{Average Veliger Growth Rate (}\mu \text{m}^2 \text{ day}^{-1})$$

$$A_1 = \text{Initial Average Veliger Shell Area (}\mu \text{m}^2)$$

$$A_2 = \text{Final Average Veliger Shell Area (}\mu \text{m}^2)$$

$$t = \text{Time (weekly)}$$

Figure 2.10. Determining shell area of surviving veligers using Image J. The boundary of the shell was determined and drawn by hand using the polygon tool in Image J.
2.3 - Results

Graphs showing the trend in percent survivorship (Figure 2.11) and average veliger growth (Figure 2.12) as a comparison between Lake Tahoe (FLTW) and Lake Mead (FLMW) waters are displayed below along with a summary table (Table 2.2) of results. The control treatment for this assay was the FLMW since quagga mussels have successfully established in this environment. All measurements are displayed as an average of all veligers used in the assay.

Table 2.2. Veliger survival and growth rate comparing Lake Tahoe and Lake Mead treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth Rate (μm² week⁻¹)</th>
<th>Survivorship (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLMW</td>
<td>0.076</td>
<td>44</td>
</tr>
<tr>
<td>FLTW</td>
<td>0.083</td>
<td>24</td>
</tr>
</tbody>
</table>

Experimental results show that percent survivorship decreased with time and was higher for veligers maintained in FLMW than for FLTW (Figure 2.11). Each data point represents the percentage of the original specimens remaining alive at the specified measurement time. Both treatment groups exhibit 100% survival for the first seven days of the assay. Survivorship for the veligers maintained in Lake Mead water remained at 100% until sometime after observations on Day 12. Day 14 observations showed a slight drop in survivorship. It continued with a slow decline until Day 26 when the rate of survivorship decreased by the greatest amount (30%) followed by only a slight decrease till Day 28. The final survivorship for Lake Mead was 44%. Survivorship for veligers maintained in Lake Tahoe water began to decline earlier (at Day 9) than veligers in Lake Mead water with a gradual decrease in survivorship for the remainder of the 28 days. The
final survivorship for Lake Tahoe was 24%. The overall shape of the survivorship data for both treatments follows a declining second order polynomial trend.

The four living behaviors observed during the assay are considered to represent a healthy and active veliger. These observations were only used to determine living versus deceased veligers. No other analysis was completed. Veligers were also observed to determine their life stage. Using the growth pictures take on Day 28, it was determined that the size of the veligers was comparable to its life stage (the largest shell sizes were of those veligers that had matured to the plantigrade stage). No other additional analysis was completed.

![Figure 2.11](image.png)

**Figure 2.11.** Average rate of survival comparing Lake Tahoe versus Lake Mead treatments. Error bars represent the standard error within the sample size where n=5.
Figure 2.12. Average area of shells for surviving veligers comparing Lake Tahoe versus Lake Mead treatments. Asterisk above Day 7 indicates a significant difference. Error bars represent the standard error within the sample size where n=5.

Average shell size increased at a similar rate throughout the course of the assay for both treatments (Figure 2.12). Overall growth was approximately linear. The growth of the veligers maintained in both Lake Mead and Lake Tahoe water gradually increased over the course of the 28 day assay. The average growth rate over the course of the assay was 0.076 µm² week⁻¹ for the FLMW group and 0.083 µm² week⁻¹ for the FLTW group. Error bars overlap from Day 14 to Day 28 suggesting that there was no significant difference in veliger size between the two treatments.
Both pH and ammonia in the waste water remained constant throughout the assay. Ammonia remained below 1ppm throughout the course of the assay. The pH ranged between 7.0 and 8.0 for the Lake Tahoe treatments and 7.5 to 8.0 for the Lake Mead treatments.

2.3.1 - Statistical Analysis

One-way statistical analysis was completed on all data to determine the effect of water type on survivorship and growth. The survivorship and growth date was analyzed by a means comparison, comparing the pairs by a student’s t-test. Survivorship was compared from Day 7 to 28. Statistically the data was different indicating that survivorship was affected by water type. The growth data was only statistically different at Day 7 indicating that during the first week growth was affected by water type.

2.4 - Discussion

Results from the survivorship and growth assays comparing FLMW to FLTW show that while survivorship in the Lake Tahoe treatment was lower, the growth rate for surviving veligers was similar between the two treatments. The statistical difference of growth between Day 0 and Day 7 can likely be attributed to the adjustment of the veligers from their original environment (Lake Mead) to FLT W. Within the next seven days, the FLT W growth rate increases. By the final day of the assay, both treatments had similar shell size.

The slight decline in growth at Day 21 of the Lake Mead veligers can be contributed to two limitations of the experiment. First, the measurement of the veligers using Image J can account for some discrepancy in area since the program is converting
pixels to $\mu m^2$. Secondly, the veligers sometime do not sit completely flat to the petri dish surface due to the shape of their shell. This caused some veligers to be at an angle when the photographs were taken, leading to fluctuations in the veligers’ measured size.

Comparing the data points and error bars between Day 14 and Day 21 shows that while the average shell area at Day 21 appears to decrease, the data point still remains within the range of the standard error. While it is unrealistic to believe that the veligers decrease in shell size, it is because of these two challenges in measuring veliger shells that at times growth appeared to decrease.

As stated previously, calcium is considered to be the key limiting factor in determining the success of establishment in an ecosystem (Whittier et al., 2008). Studies of quagga mussels in aquatic systems in the eastern United States have shown similar results. A study from Jones and Ricciardi (2005) showed that populations of quagga mussels in the St. Lawrence River appeared to be limited to calcium concentrations higher than 12 ppm.

Baldwin et al. (2012) took this study further to look at a variety of inland waters susceptible to secondary invasion using quagga mussels at multiple life stages. They used treatments ranging in calcium concentration from 2.03 to 32.07 ppm (Baldwin et al., 2012). This study showed that quagga mussel veligers were able to survive and grow to the pediveliger stage at calcium concentrations greater than 12 ppm. Their survival was higher as calcium concentrations increased.

As a result of the low level of calcium in Lake Tahoe (approximately 12 ppm) versus the high level of calcium in Lake Mead (approximately 70 ppm) dissolved calcium is considered to be the limiting factor of survivorship for veligers in the FLTW treatment.
However, there are other dissimilarities between the water chemistry of the two Lakes including nitrate (NO$_3^-$), magnesium (Mg), sodium (Na), and potassium (K). Lake Tahoe exhibited lower values (nearly 10% of Lake Mead for NO$_3^-$, Mg, Na and 35% for K) in these additional four parameters (Table 2.1) than the Lake Mead samples analyzed. While nitrate, sodium, and potassium might also be limiting factors to veliger survivorship, literature for zebra mussels indicates that a favorable Ca:Mg balance is imperative to survival (Dietz et al., 1994).

In a study of zebra mussels from Dietz et al. (1994), they found that the mussels will survive in a low calcium environment provided that there is magnesium (Mg) “in minimal amounts”. Dietz et al. determined that an environment with sufficient calcium could support a large population of zebra mussels with a minimum of 0.1 mM (24.3 mg L$^{-1}$) magnesium (Dietz et al., 1994). The average magnesium level in the outflow from Lake Mead from 2001 to 2009 was 27.0 ppm (Holdren and Turner, 2010) and the average measured magnesium in the Lake Mead water sampled for these assays was 21.50 ppm (Table 2.1). Lake Mead magnesium levels are considered adequate for quagga mussels.

The average magnesium level of the samples acquired at Lake Tahoe is considerably lower at 2.42 ppm (Table 2.1). Studying magnesium levels (and other nutrient constituents besides calcium) was outside the scope of this study. However it is an important factor for quagga mussel establishment in calcium limited environments. It is recommended that further research is needed to determine if the low levels of magnesium in Lake Tahoe would have an effect on quagga mussel establishment.

The results presented in this Chapter provide a baseline comparison of survivorship and growth rate potential in Lake Tahoe and Lake Mead. This baseline
information was used not only for the following assays but can be applicable to other low calcium environments. The results of this Chapter show that dissolved calcium levels are not a limiting factor in the ability of a veliger to grow. However, lower calcium might attribute to the lower survivorship. To test this further, an additional assay was conducted using five water treatments. The treatments included three conditions in which calcium was added to FLTW to three increments of increasing calcium concentrations.
CHAPTER 3 - CALCIUM AMENDED COMPARATIVE SURVIVAL
AND GROWTH ASSAY

3.1 - Introduction

Based on the data provided in Chapter 2, it is assumed that calcium deficiency is a limiting factor for veliger survivorship in the Lake Tahoe environment. To test the hypothesis of this Chapter, additional survival and growth assays were run with incrementally higher levels of dissolved calcium in the FLTW (Table 3.1). The results of this assay performed in this Chapter will add to the understanding of the importance of calcium to quagga mussel establishment.

The target levels of amended calcium for the FLTW were chosen to imitate natural occurrences of elevated calcium in Lake Tahoe and test how the varying levels affected veliger survival and growth. The three amended levels were selected using information from literature reviews (Hincks and Mackie, 1997) and analysis of specific characteristics and parameters within the Lake Tahoe system. The three additional calcium concentrations chosen were: 20 (FLTW-Elevated), 25 (FLTW-Clam Beds), and 32 (FLTW-Maximum) ppm dissolved calcium. These names and target calcium concentrations were designated for organization of the assay. FLTW-Elevated at 20 ppm is slightly above the calcium concentration in the main parts of the Lake, the Tahoe Keys, and similar to elevated areas in the region. FLTW-Clam Beds at 25 ppm is the average calcium concentration found in the invasive Asian clam beds, a possible place of quagga mussel establishment. FLTW-Maximum at 32 ppm calcium is above literature thresholds for maximum growth rate of juvenile zebra mussels (Hincks and Mackie, 1997).
Baldwin et al. (2012) is one of few papers where quagga mussel veligers were raised in a laboratory setting. They focused their study on inland tributary rivers and lakes in northern New York State. The differences between the two studies are discussed in the Section 3.4 of this Chapter.

Questions

Will Lake Tahoe water with elevated levels of calcium be able to better support veliger quagga mussels and promote growth similar to the growth rates in Lake Mead water? Will the additional calcium also aid in the veligers ability to survive in the Lake Tahoe water?

Hypothesis

Growth rate and survivorship will increase with increasing levels of dissolved calcium.

3.2 - Methods

Treatment water was collected and analyzed by the same methods described in Chapter 2, Section 2.2.1. Lake Tahoe water was delivered to Las Vegas one week prior to the start of the experiment and stored in aerated, closed buckets. Lake Mead water was collected at the time of veliger collection and was stored with aeration for the duration of the experiment. Samples were analyzed by a third party water analysis laboratory for actual calcium concentrations.

The amended calcium treatment water was made in one large batch within one week of the start of the experiment by adding powdered calcium (Calcium Chloride Dihydrate, CaCl\textsubscript{2} 2H\textsubscript{2}O from Fischer Scientific) to FLTW to achieve the estimated concentration (Dietz et al., 1994). Calculations used to determine the required amount of
CaCl$_2$ 2H$_2$O to be added to the FLTW assumed that the FLTW started with a calcium concentration of 11 ppm. Due to this assumption and errors in measurements due to the very small amount of powered calcium required, actual calcium concentrations are varied from the target level (Table 3.1). Amended treatments were kept aerated and stored in plastic buckets for the duration of the experiment (Figure 3.1).

**Table 3.1.** Calcium (Ca) concentration of treatment waters. *Target calcium levels were designated for organizational and naming purposes. For example the calcium concentration varies in Lake Mead so the average of 76.0 ppm was chosen as a reference point. For the amended FLTW waters, the target calcium concentrations were used as a reference point. More important than their naming is that the actual calcium concentrations determined during water analysis give three different levels of calcium similar to those that can be found naturally in Lake Tahoe.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (ppm)</th>
<th>Target*</th>
<th>Actual Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLMW</td>
<td>76.0</td>
<td>69.0</td>
<td></td>
</tr>
<tr>
<td>FLTW</td>
<td>12.0</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>FLTW-Elevated</td>
<td>20.0</td>
<td>20.7</td>
<td></td>
</tr>
<tr>
<td>FLTW-Clam Beds</td>
<td>25.0</td>
<td>29.3</td>
<td></td>
</tr>
<tr>
<td>FLTW-Maximum</td>
<td>32.0</td>
<td>34.0</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.1. Lake Tahoe water with additional calcium aerated and stored in laboratory. These additional treatment waters were made just prior to the start of the experiment.

Following the same procedure presented in Chapter 2, Section 2.2.4-5 a survivorship and growth assay was conducted using all five water types (FLMW, FLT2W, FLT2W-Elevated, FLT2W-Clam Beds, and FLT2W-Maximum). Veligers were collected following the same procedures in Chapter 2, Section 2.2.2 in mid-August. The only difference in procedure was that four replicate petri dishes with 5 veligers were used for each treatment (20 veligers per treatment as opposed to 25 in Chapter 2). The average water temperature at Callville Bay in August is 29 °C (Resorts, 2014). Results were analyzed as a comparison across the five water treatments.

3.3 - Results

Graphs showing the trend in percent survivorship (Figure 3.2) and average veliger growth (Figure 3.3) as a comparison of all five treatments are displayed below along with a summary table (Table 3.2) of results. The control treatment for this assay was again the
FLMW since quagga mussels have successfully established in this environment.

Measurements are displayed as an average of all veligers used in the assay.

Table 3.2. Veliger survival and growth rate comparing treatments of varying calcium levels after 28 Days. Refer to Table 3.1 for the dissolved calcium concentrations associated with the treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth Rate (μm² week⁻¹)</th>
<th>Survival Rate (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLMW</td>
<td>0.079</td>
<td>95</td>
</tr>
<tr>
<td>FLTW</td>
<td>0.119</td>
<td>30</td>
</tr>
<tr>
<td>FLTW-Elevated</td>
<td>0.075</td>
<td>40</td>
</tr>
<tr>
<td>FLTW-Clam Beds</td>
<td>0.071</td>
<td>50</td>
</tr>
<tr>
<td>FLTW-Maximum</td>
<td>0.076</td>
<td>65</td>
</tr>
</tbody>
</table>

Figure 3.2. Average rate of survival comparing low, high, and amended calcium treatments. Error bars represent the standard error within the sample size where n=5.
Survivorship of the veligers in this assay shows an apparent dependence on dissolved calcium (Figure 3.2). All treatment groups showed declining survivorship with time; however, the rate of mortality was inversely dependent on calcium concentration in the culture water. Each data point represents the percentage of the original specimens remaining alive at the specified measurement time. Veligers maintained in the high calcium treatment (FLMW) remained at 100% survival through Day 16 and has the highest final survival rate at 95%. The FLTW-Maximum treatment lost a few individuals within the first four days and remained consistent until another drop in survivorship after Day 16. The final survival rate for veligers in FLTW-Maximum water was 65%. The FLTW-Clam Beds treatment followed a similar trend to FLTW-Maximum with fewer surviving and a final survival rate of 50%. While more veligers in the FLTW-Clam Beds treatment survived in the first few days compared to the FLTW-Maximum treatment, their percentages overlap at Day 7 followed by a drop in FLTW-Clam Beds below FLTW-Maximum. The FLTW-Elevated and FLTW treatments both drop consistently at days of readings. The veligers in the FLTW water had slight more individuals surviving in the first four days than FLTW-Elevated but then drops below the FLTW-Elevated percentage. These two treatments once again overlap at Day 21 with FLTW proceeding to lose more individuals towards the final days. Slightly more veligers survived in the FLTW-Elevated treatment (40%) compared to FLTW (30%). The overall shape of the survivorship data for all five treatments follows a declining second order polynomial trend.
Figure 3.3. Average area of shells for surviving veligers comparing low, high, and calcium amended treatments. Error bars represent the standard error within the sample size where n=5.

Overall growth increased for surviving veligers and shell size increased overall throughout the course of the assay (Figure 3.3). There was only a slight decline in FLT W at Day 7 and FLT W-Clam Beds at Days 7 and 14. Again this was most likely again due to limitations for photography and measuring process. Resulting growth rates were: 0.079 µm²week⁻¹ for the high calcium environment, 0.119 µm²week⁻¹ for low calcium, 0.075 µm²week⁻¹ for FLT W-Elevated, 0.071 µm²week⁻¹ for FLT W-Clam Beds, and 0.076 µm²week⁻¹ FLT W-Maximum. The growth rate appears to be independent of calcium indicating that the threshold for calcium needed by the veligers to grow was met. The growth rates for all treatments are very similar. Error bars overlap suggesting that there was no significant difference in veliger size between the five treatments.
The variation in initial size of the veligers at the start of the growth assay can be contributed to three limitations of the experiment. First the veligers were hand selected and approximated for their size. Secondly, the measurement of the veligers using Image J can account for some discrepancy in area since the program is converting pixels to $\mu m^2$. Finally, the veligers sometime do not sit completely flat to the petri dish surface due to the shape of their shell. This caused some veligers to be at an angle when the photographs were taken, leading to fluctuations in the veligers’ measured size. Although there is a difference in initial veliger size, the difference is by one hundredths of a squared micron and is very small compared to the overall growth of the veligers.

Both pH and ammonia in the treatment water again remained constant throughout the assay. The pH ranged between 7.0 and 8.0 for the Lake Tahoe and the three calcium amended treatments and 7.5 to 8.0 for the Lake Mead treatments. Ammonia remained below 1ppm throughout the course of the assay.

### 3.3.1 - Statistical Analysis

Statistical analysis was completed on all data to determine the effect of water type on survivorship and growth. It was first determined that the data did not represent a normal distribution. Therefor a Wilcoxon rank score was used to compare the means for both the survivorship and growth date. Survivorship was compared from Day 2 to 28. Statistically the data was different indicating that survivorship was affected by water type. The growth data was not statistically different indicating that growth was not affected by water type.
3.4 - Discussion

The results of the survival and growth assay with the amended calcium treatments mirrored the results in the previous chapter and partially support the hypothesis. As environmental calcium increased, so did the survival rate. Again we see that growth rate is very similar amongst the five treatments. Despite the lower survivorship, those veligers which survive in the low calcium treatments grow at a similar if not greater rate than the higher calcium treatments.

The data can be correlated to the initial findings comparing Lake Tahoe and Lake Mead presented in Chapter 2. Growth rate was similar between both assays. Overall survivorship increased between the assay in Chapter 2 and the assay presented in Chapter 3. Survivorship for the veligers in the FLMW treatment increased by 51% while the veligers in the FLTW treatment increased by only 6%. This change in survivorship can be accounted to two factors: the improvement of laboratory technique and initial size of the collected veligers. Veligers are especially fragile and require delicate and minimal handling. As time went on laboratory skills, especially veliger handling, improved. Since the assay in Chapter 3 was done after Chapter 2, this assay was done with softer handling and better overall techniques. Also the initial size of the veligers collected for the calcium amended assay was larger. For the FLMW treatment the veligers were approximately 4,000 µm² (average of total shell areas) and those veligers collected for the FLTW treatment were approximately 10,000 µm² larger. The larger size indicates a healthier and more mature veliger at the start of the assay. While survivorship greatly increased for the veligers in the FLMW, the environmental conditions were still not ideal for the veligers in the FLTW despite improved handling and an increase in initial size.
There are a few differences between the study presented in this Chapter and the study from Baldwin et al. (2012). First, Baldwin et al. spawned adult quagga mussels to then raise the veligers from zygote stage to pediveliger stage (Baldwin et al., 2012). While this procedure allowed for veligers to be studied at a younger age than represented in this Chapter, it did not simulate the transferring of veligers from one aquatic system to another. This is pertinent to the study of the spread of invasive species since there is high likelihood that veligers could be transported from one lake to another by recreational vehicles.

Secondly, Baldwin et al. only observed veligers for 10-14 days (Baldwin et al., 2012). In their conclusions they suggest that additional studies be completed with longer term laboratory studies to better understand the importance of calcium on the species (Baldwin et al., 2012). The study represented in this Chapter observed veligers for 28 days to maximize the observation time and also to limit the negative effects of raising the species in a laboratory setting.

Finally, Baldwin et al. fed their test veligers cultured *Nannochloris* sp. daily at an approximate concentration of $5 \times 10^8$ cells L$^{-1}$ (Baldwin et al., 2012). In the study in this Chapter, veligers were not supplemented with additional food. Only food which was present in the treatment water was available for the veligers’ consumption. This better simulated a natural environment and displays the actual food type and availability in the natural waters.

The conclusion from this study is that calcium is a limiting factor in Lake Tahoe. However, with varying levels of calcium in different areas of the Lake, there is the
possibility that if veliger quagga mussels were released they would have the ability to survive and grow.
CHAPTER 4 - SETTLEMENT ASSAY COMPARING THREE WATER TYPES

4.1 - Introduction

Dreissenids are the only freshwater bivalves which are epifaunal, meaning they live upon or on top of all types of solid substrate (Claudi and Mackie, 1994). This includes, but not limited to: rocks, organic matter, boats, intake structures, concrete, metal, glass and plastic surfaces. When quagga mussel veligers find a suitable surface and environment, they attach (settle) and continue to metamorphose to adulthood. Therefore, understanding settlement rates of quagga mussels provides information on the likelihood of their growth and success of colonization in the water that they invade.

In this study, veligers were raised under semi-natural laboratory conditions in aquariums and allowed to settle or attach. The number of settled veligers was counted and compared between three water types (FLMW, FLT W, and FLT W-Clam Beds). The percent of veligers which settle after the 30 day assay will help indicate if conditions are met in each treatment water to promote establishment.

Frischer et al. (2005) also completed a settlement study using zebra mussel veligers to compare settlement in Lake Champlain and Lake George. This trial only observed the veligers after seven days. The assay presented in this chapter was conducted for 30 days, a significantly longer period of observation than the previous veligers settlement studies.

Questions
Can quagga mussel veligers settle in the low calcium water of Lake Tahoe? Is the final number of settled veligers different between the low calcium water (FLTW) and the high calcium water (FLMW)? By adding calcium to Lake Tahoe water, will settlement increase?

**Hypothesis**

Less veligers will settle in the low calcium Lake Tahoe water compared to that of the high calcium Lake Mead water while settlement will increase with additional calcium in the Lake Tahoe water.

**4.2 - Methods**

Veligers were collected following the same procedures in Chapter 2, Section 2.2.2 in mid-July. The average water temperature at Callville Bay in July is 27 ºC (Resorts, 2014) A total of 15 250 mL samples were collected. Samples were kept unfiltered to prevent removing large veligers from the sample population. Under laboratory conditions, all samples were combined into one large plastic beaker (~4,000 mL total of samples) and carefully mixed to ensure that veligers were equally distributed. The combined samples were then immediately poured into 12 Imhoff cones (Figure 4.1) with approximately 250 mL of sample poured into each cone. The cones were kept at ambient laboratory temperature (approximately 20 ºC) for two hours. The cones allowed the veligers to settle to the bottom while swimming predators remained in suspension. After two hours of settlement, 2 mL aliquots (using a Mohr pipette) were taken from the bottom of three randomly selected cones and observed under cross polarized light at 20x magnification (using a Nikon SMZ1000 stereomicroscope) to count the total number of
veligers per aliquot. It was determined that on average there were 500 veligers per 2 mL of sample in each individual cone.

![Image of Imhoff cones with veligers settling](image)

**Figure 4.1.** Veligers settling in Imhoff cones at ambient laboratory temperature for two hours.

Settlement tests were conducted using five (5 gallon) aquarium tanks each holding 16 L of water. Each tank was filled with 16 L of one of three water types. Two tanks were filled with FLMW, two FLTW, and one FLTW-Clam Beds (see Sections 3.1 and 3.2 for water description). Once again these treatment waters were analyzed for actual calcium concentrations (Table 4.1). Water from Lake Tahoe was transported to the Las Vegas laboratory one week prior to the start of the experiment and the tanks for the FLTW and FLTW-Clam Bed treatments were prepped with aeration, lighting, and exposed to ambient temperature one day prior to Day 0 (start of the settlement experiment). Lake Mead water was collected at the time of the veliger collection. The FLMW treatment tanks were prepped to the same standards upon return to the laboratory.
while veliger samples settled and population size was counted. Water collection and analysis followed the same procedures described in Chapter 2, Section 2.2.1.

The entire bottom of each tank was covered with 32 individually numbered 2x2 inch glass tiles to facilitate attachment (Figure 4.2). Each tile was lined on one side with black electrical tape to allow for easy removal from the bottom of the glass aquarium. Tiles in all five tanks were laid out in the exact same order. Due to size restrictions and to ensure that the entire bottom of the aquarium was lined, tiles numbered 7-8, 15-16, 23-24, and 31-32 were overlapped with the higher numbered tile overlapping the lower numbered tile by approximately half of an inch. Sheets of paper with a grid labeling each tile number was placed underneath the aquariums.

Veligers were collected from the bottoms of 10 settling cones for use in the experiment with two randomly selected cones assigned to each tank. A combined total of 14 mL of fluid was pipetted from the bottom of the two cones and added to each tank, introducing approximately 3500 veligers per tank. The tanks were aerated continuously, and exposed to LED aquarium lighting on timers to maintain a 12 hour light/dark cycle (Figure 4.3). Fresh water (FLMW, FLTW, or FLTW-Clam Beds) was added regularly to compensate for evaporative loss. Ammonia and pH was tested prior to each water addition using the procedure described in Chapter 2, Section 2.2.1.

Settlement was approximated by counting the number of veligers attached to the glass slides. Every few days, a randomly selected tile was removed from the tank and placed in a petri dish with 5 mL of the appropriate water type. The water in the petri dish was swirled gently to remove any non-attached veligers from the glass slide. Tiles were observed using cross polarized light with a Nikon SMZ 1000 stereomicroscope to count
the number of attached veligers. After observation, tiles were returned to their original location in each tank along with the water from the petri dish. These daily observations were only done to ensure that veligers were attaching during the course of the assay. On Day 30 (final day of the experiment), all 32 tiles were removed from each tank and the attached veligers were counted following the same procedure described above. Only veligers attached to the tiles were able to be counted. The data does not represent veligers that might have attached to the sides of the aquariums. There is not a logical way to collect this data since the veligers are still microscopic. Throughout the course of this study, pictures were taken of veligers for educational purposes only.

**Table 4.1.** Calcium (Ca) concentration of treatment waters. *See note on Target nomenclature in the caption of Table 3.1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (ppm)</th>
<th>Target* Ca</th>
<th>Actual Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLMW</td>
<td>76.0</td>
<td>80.2</td>
<td></td>
</tr>
<tr>
<td>FLMW</td>
<td>76.0</td>
<td>82.3</td>
<td></td>
</tr>
<tr>
<td>FLTW</td>
<td>12.0</td>
<td>12.8</td>
<td></td>
</tr>
<tr>
<td>FLTW</td>
<td>12.0</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>FLTW-Clam Beds</td>
<td>25.0</td>
<td>31.5</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.2. Glass tiles lining the bottom of the settlement tanks showing the numbering of the tiles.

Figure 4.3. Settlement tanks in separate secondary containment (per laboratory containment protocols) with lighting and aeration.
4.3 - Results

A table summarizing the results of the settlement assay is provided below (Table 4.2). Settlement rates after thirty days were highest for the high calcium treatment (FLMW) with 10.31% settled. Settlement decreased with decreasing calcium levels (4.66% for FLTW-Clam Beds and 1.14% for FLTW). The number and percent of veligers settled was corrected for survivorship using the data from Chapter 3 (FLMW-95%, FLTW-30%, FLTW-Clam Beds-50%). Corrected settlement percentage assumes that 100% of the veligers survived the course of the experiment and all had equal opportunity to settle. Through observations of the settlement tiles, veligers which attached appeared healthy and grew throughout the thirty day experiment (see Figure 4.4 for an example of healthy, settled veligers on Day 30).

The distributions of veligers on the tiles appeared to be random. From observations, it appeared as if veligers liked to attach to the sides of the glass slides. However, that number was not kept separate and no analysis was completed.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Settled Counted</th>
<th>Settled Percent Uncorrected</th>
<th>Settled Percent Corrected</th>
<th>Avg. Ca Conc. (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLMW</td>
<td>361</td>
<td>10.31</td>
<td>10.86</td>
<td>81.25</td>
</tr>
<tr>
<td>FLTW</td>
<td>40</td>
<td>1.14</td>
<td>3.81</td>
<td>12.65</td>
</tr>
<tr>
<td>FLTW-Clam Beds</td>
<td>163</td>
<td>4.66</td>
<td>9.31</td>
<td>31.50</td>
</tr>
</tbody>
</table>
Figure 4.4. Veligers attached to a glass tile during settlement assay. Mussels are shown using siphons to filter water. (Nikon SMZ1000 stereomicroscope at 60 magnification)

4.4 - Discussion

Settlement is an important indicator of whether or not quagga mussels can become successfully established in a system. Successful settlement indicates that the required parameters have been met to allow the veliger to metamorphose to an adult. The results of this study demonstrate that veligers can settle in a low calcium environment and that calcium concentration impacts the percentage of introduced veligers that will settle. The Lake Tahoe treatment corrected to 100% survivorship experience 3.81% of veligers settled. In natural waters and with a larger population size, the potential is there for a significant number of veligers to successfully settle in the low calcium environment.
A similar study with zebra mussel veligers compared settlement in water from water from Lake George with no manipulation and amended (10.65 and 30.89 ppm Ca respectively). This study only observed veligers after seven days and used approximately 15,000 veligers added to triplicate 20 gallon aquariums with 20 removable standard glass microscope slides lining the bottom (Frischer et al., 2005). Calcium supplementation did improve settlement in the Lake George treatment from 0.63 percent in the non-manipulated water to 32.8 percent in the amended treatment (Frischer et al., 2005).

For the settlement experiment presented in this Chapter, three major changes were made from the Frischer et al. (2005) experiment. First, it was determined for this settlement assay that one week of exposure to the treatment water was insufficient to portraying accurate results. Using previous trial experiments done in the laboratory to understand the basic needs of veligers, we felt that the veligers require about one week to adjust to moving from Lake Mead to the other treatments. Second, we decreased the number of veligers per aquarium to minimize competition. Thirdly, the entire bottoms of the aquariums were covered in glass slides. Based on the procedure presented by Frischer et al., it is unclear whether they completely lined the bottom of the aquarium as well.

A few non-ideal situations occurred due to the design of this study. First, to obtain the largest population possible, the estimated number of veligers included living and deceased. Second, by only counting the tiles on the bottom of the aquarium, veligers which might have attached to the sides of the aquariums were not accounted for.

Previous studies have concluded that the release of a small number of veligers by recreational vehicles would not likely facilitate an establishing colony due to the veligers becoming diluted and suffering high mortality rate in transit (Johnson et al., 2001;
Sprung, 1993). It is more likely that adults would be transferred with the possibility of reproduction and establishment. However it is likely that multiple delivery methods would need to take place in order for a successful establishment to occur (Johnson et al., 2001). In this case the combination of some adults plus veligers from recreational vehicles would provide a variety of life stages and create a surviving colony.

Johnson et al. (2001) determined the approximate number of zebra mussel veligers per live well in recreational vehicles leaving Lake St. Clair in Michigan. The live wells on average contained 25 ± 22 veligers per liter with the average live well about 65 liters in volume (Johnson et al., 2001). They admitted that this could be an underestimate depending on the time of year and veliger quantity in the lake. The situation of the live wells with veligers suspended in the water is similar to the settlement assay conducted in this Chapter. Like in the settlement tanks, a few of the healthiest veligers might survive transportation in a live well to a previously uninhabited lake.
CHAPTER 5 - CONCLUSION

5.1 - Summary of Results

Based on the results found in the preceding Chapters, it was determined that while dissolved calcium concentration affects the survivorship of quagga mussel veligers, the surviving veligers grow at a similar rate. The growth rate is independent of calcium concentrations. Growth rate between the two assays was similar, confirming that calcium was not a limiting factor to growth.

Survivorship, however, was different between the two assays, with survivorship increasing in the FLMW and FLTW treatments in the assay in Chapter 3. This can be accredited to improved laboratory techniques with experience. While the survivorship was not the same in the two assays, both assays did portray a correlation between calcium and survivorship indicating a direct relationship.

The number of veligers which settled also increased as calcium concentrations were increased. This indicates that the environmental conditions required by the veligers were met. While the settlement was very low for the FLTW treatment, the veligers which settled appeared healthy and grew.

The data indicates that there is the high possibility of quagga mussel establishment in Lake Tahoe. It is recommended that boat inspections and washings be continued to help prevent quagga mussel introduction. Other lakes and streams in the western United States should also take the same precautions, despite having lower calcium concentrations.
5.2 - Recommended Future Research

The studies presented in this thesis add to current understanding of quagga mussel veliger growth and sensitivity to calcium in waters of the western United States. The results of these studies suggest gaps in the current knowledge base that should be addressed in future studies. First, it is suggested that future experiments use treatments containing varying levels of both calcium and magnesium. Magnesium might also play an important role in veliger development and a better understanding of the levels required is pertinent. Secondly, while these studies only observed behaviors and life stages, there is the potential to use the knowledge gained in these assays to complete a study on just veliger behaviors and life stages (including a correlation to shell size). Thirdly, veliger shells in this study were not analyzed for thickness or fragility. An additional study could be conducted to analyze the physical shell strength compared between systems of varying calcium concentration. Finally, these experiments are designed in a way that they may be duplicated using treatment water from any river or lake system. This would provide data specific to a system and allow for more specialized management and prevention.
APPENDIX 1. EXPERIMENTAL CLEAN UP

Disposal of water and material which has come into contact with an invasive species requires a specific protocol to prevent further spread of the species (Link, 2010). All waste water was chlorinated (50 ppt) and stored in 20 L plastic buckets for a minimum of 24 hours. The water was then de-chlorinated with sodium thiosulphate and checked for neutral pH prior to disposal. Reusable laboratory and sampling equipment were cleaned in 20 L plastic buckets containing diluted chlorine (50 ppt) soaking for a minimum of 24 hours and then rinsed in triplicate with hot tap water and DI water. Disposable equipment (e.g., petri dishes) was left to dry for a minimum of 30 days before being discarded in regular laboratory waste. (Link, 2010)
APPENDIX 2. STATISTICAL ANALYSIS

Statistical analysis was completed on all data using JMP (Release 5.0.1.2, SAS Institute Inc.) with \( p = 0.05 \), corresponding to a 95% confidence level. For assays with two sets of data, a student t-test was used to compare the means. For assays with more than two sets of data, the distribution was first tested for normality. If the data had a normal distribution, an analysis of variance (ANOVA) was performed to compare the means. If the data did not have a normal distribution, a Wilcoxon rank score was used to compare the means. Error bars in all figures display standard error of the data.
APPENDIX 3. BROCHURE USED TO EDUCATED BOATERS AND THE PUBLIC ON BOAT INSPECTIONS AND THE SPREAD OF INVASIVE SPECIES IN NEVADA (PAMPHLET RECEIVED IN PERSON FROM NEVADA DEPARTMENT OF WILDLIFE)
HELP PROTECT NEVADA’S WATERS
You can stop the spread of aquatic hitchikers!
Clean, Drain and Dry ... Everytime and Everywhere.
- Waders
- Fishing Gear
- Canoes/Kayaks
- Bait Buckets
- Boats
- Fishing Poles

If it gets wet, it could harbor Aquatic Invasive Species. Inspect Everything!

SELECTED AQUATIC INVASIVE SPECIES

<table>
<thead>
<tr>
<th>Quagga Mussels</th>
<th>Eurasian Watermilfoil</th>
<th>New Zealand Mussels</th>
<th>Zebra Mussels</th>
</tr>
</thead>
</table>

INSPECT AND DECONTAMINATE YOUR WATERCRAFT AND EQUIPMENT
Here are some methods to minimize your chances of accidentally transporting invasive species. By following these steps you can help protect your valuable fishing and boating resources for the future:

EVERYTIME - EVERYWHERE
- **CLEAN** Remove all mud, plants and animals from every part of your boat, trailer and equipment.
- **DRAIN** Before you leave the recreation area, eliminate all water from your boat, including its live-wells, ballast, hull and engine-cooling water.
- **DRY** Allow time for your boat to completely dry before you launch in any other waters. This amount of time may vary depending on humidity and temperature. In the summer, your dry time should be at least seven days.

*Diagram of watercraft and equipment components:*
- Trailer
- Anchor
- Dock Lines
- Live Wells Bilge
- Vehicle
- Rollers
- Hull
- Axle
- Motor
APPENDIX 4. NEVADA DEPARTMENT OF WILDLIFE SPECIAL COLLECTION PERMIT

NEVADA DEPARTMENT OF WILDLIFE
SPECIAL LICENSE/PERMIT

Date Issued 9/4/2012  License Type Scientific Collection Permit

Entity / Licensee / Permittee Name Thaw Melissa N – Desert Research Institute

Mailing Address 755 E Flamingo Rd

City Las Vegas  State NV  ZIP 89119

- Same -

Street Address

City - Same -  State  ZIP

Tax ID/SSN  Date of Birth 08/09/82  Sportman’s ID

License Class 22.92  Agent No. 1950  Issued by jgm  Fee $ 100.00

License/Permit Valid From September 4, 2012 through December 31, 2014

S 36161

--- Special Conditions ---
All applicable sections set forth in the Nevada Administrative Code (NAC) and Title 45 of the Nevada Revised Statutes (NRS) shall apply.

- Authorizations and Conditions Attached -
- Period of Collection Activities: See Condition #4 -
- Activity Report(s) Due: 01/30/2014; 01/30/2015 -
PERMITTEE:
Melissa Thaw
Desert Research Institute
755 E Flamingo Rd
Las Vegas NV 89119

Permit No.: S36161
Date Issued: 9/4/2012
Date Effective: 9/4/2012
Period of Sampling: See Condition #4
Expiration Date: 12/31/2014
Annual Report Due: 1/30/14 & 1/30/15
Fed. Permit No.: NA

SCIENTIFIC COLLECTION PERMIT NO. S36161

In compliance with the conditions listed below and pursuant to provisions of NRS 503.597 & 503.650, the permittee, each permit year during the designated sampling period, is authorized to:

a. Capture live specimens, transport and maintain alive in captivity, 1000 Quagga mussel (Dreissena bugensis).

Prior to any sampling or collection activity at Lake Mead, Permittee must notify NDOT Southern Region biologist Jon Sjoberg @ (702) 486-5127 ext 3300 or sjoberg@ndow.org.

Note: Permittee must follow the protocol for Quagga Mussel for transport and containment.

CONDITIONS:

1. A copy of this permit and any permits required by the U.S. Fish and Wildlife Service must be in the possession of the permittee and any authorized collectors while conducting collection/salvage activities. The permittee must comply with all terms, conditions and restrictions of the federal permit. This permit is invalid for the taking, collection, or salvage of migratory birds, threatened or endangered species, absent any permit required by the Service for that activity.

Activities authorized under this permit to collect and/or possess wildlife, parts thereof, or their progeny, shall be in compliance with all other state and federal regulations.

2. Authorized Sampling Area: Lake Mead in Clark Co.

This permit does NOT authorize trespass and/or collecting activities on state or federal wildlife refuges or reserves, or other public and private property without the permission from landowner or custodian.

3. Number Authorized: As indicated above.


5. Destination of Collection: Desert Research Institute, 755 E Flamingo Rd, Las Vegas, NV
6. **Annual Report:** A record will be created for each specimen (or group of specimens of a single species) taken at each site-locality. “Taken” means salvaged; captured & released; collected; banded; trapped & killed; seined; netted; snared; sacrificed; reduced to possession; etc. The following information will be recorded for each specimen taken: By date, the number of specimens of each species taken; species name; the habitat type where each specimen was taken; numeric breakdown of sex whenever possible; and a description of the location where each specimen was taken, by the following method: (Don’t use common geographic names)
- UTM Coordinates, NAD 83, Zone 11, rounded to the nearest meter.

The records must be submitted to the Nevada Department of Wildlife License Office – Scientific Collection Report, 4600 Kietzke Ln D-135, Reno, NV 89502, by 1/30/14 for 2013 “take” activities; and 1/30/13 for 2014 “take” activities. Digital reports in Excel spreadsheet (preferred) or Quattro Pro are accepted (please follow column sequence as outlined in the Department report form, 22.65-5.).

7. A copy of all pertinent research or technical papers must be submitted to the Department.

8. All specimens authorized under the authority of this permit, including offspring, are property of the State of Nevada and as such, they shall not be sold, bartered, traded, converted to personal use or otherwise disposed of without written approval of the Department, except as provided in Condition #5. This condition remains in effect indefinitely.

9. No fee may be charged to the public for the privilege to view wildlife which is held under the authority of this permit.

10. **Permit Cancellation:** A violation of a condition or stipulation is cause for the cancellation of the permit.

11. **Additional Authorized Collectors:** Dr. Kumud Acharya and authorized research assistants under the direction of the Permittee.

Julie Meadows
Program Officer I

jgm
enclosure


https://www.google.com/maps/place/Lake+Mead/@36.2610864,-114.4178569,10z/data=!3m1!4b1!4m2!3m1!1s0x80c95286ddd43a6f:0xb514b9cb7a1cb0ca.

https://www.google.com/maps/place/Lake+Tahoe/@39.0854459,-120.0450526,11z/data=!3m1!4b1!4m2!3m1!1s0x809978a1b91f1151:0x8c3f1faeaf520.


*Lake and Reservoir Management, 26*(4), 230-239.


Retrieved from


Michigan Department of Natural Resources (DNR). (2008). In Southwest Quagga Team Meeting. *Lake Huron’s food web change*. Henderson, NV


VITA

Graduate College
University of Nevada, Las Vegas

Emma Kathleen Ruhmann

Local Address
11000 South Eastern Avenue
Apartment Number 2514
Henderson, Nevada 89052

Permanent Address
1040 North Jefferson Street
Florissant, Missouri 63031

Degree
Bachelor of Science, Environmental Science
Concentrations in Chemistry and Geoscience
Saint Louis University

Publications


Thesis: Survival, growth, and settlement of *Dreissena rostriformis bugensis* veligers in low and high calcium waters

Thesis Examination Committee
  Chairperson: Michael Nicholl, Ph.D.
  Committee Co-Chair: Kumud Acharya, Ph.D.
  Committee Member: Craig Palmer, Ph.D.
  Graduate Faculty Representative: Carl Reiber, Ph.D.