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Matthew Kappel
University of Nevada, Las Vegas, threemilesdown@gmail.com

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DREISSENA ROSTIFORMIS BUGENSIS: DESICCATION OF ADULT QUAGGA MUSSELS FOUND IN LAKE MEAD AS A PREVENTIVE MEASURE AGAINST OVERLAND DISPERSAL IN THE WESTERN UNITED STATES

by

Matthew Kappel

Bachelor of Science
University of Nevada, Las Vegas
2007

A thesis submitted in partial fulfillment of
the requirements for the

Master of Public Health

Department of Environmental and Occupational Health
School of Community Health Sciences
Graduate College

University of Nevada, Las Vegas
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THE GRADUATE COLLEGE

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Department of Environmental and Occupational Health

David Wong, Ph.D. Committee Chair

Shawn Gerstenberger, Ph.D. Committee Member

Paulo Pinheiro, Ph.D. Committee Member

Steen Madsen, Ph.D. Graduate College Representative

Tom Piechota, Ph.D., Interim Vice President for Research &
Dean of the Graduate College

December 2012

ABSTRACT

***DREISSENA ROSTIFORMIS BUGENSIS*: DESICCATION OF ADULT QUAGGA MUSSELS FOUND IN LAKE MEAD AS A PREVENTIVE MEASURE AGAINST OVERLAND DISPERSAL IN THE WESTERN UNITED STATES**

by

Matthew Kappel

Dr. David Wong, Examination Committee Chair
Associate Research Professor

Dr. Shawn Gerstenberger, Examination Committee Co-Chair
Executive Associate Dean of School of Community Health Sciences
University of Nevada, Las Vegas

The expansion of civilization across all borders of the world has proven to affect major components of ecosystems. Since the introduction and establishment of the aquatic invasive species (AIS), *Dreissena rostiformis bugensis*, commonly known as the quagga mussel, into the United States there has been an extensive amount of time and money spent on controlling and preventing their expansion across the United States. The quagga mussel is of major concern because of its ability to disrupt the ecological communities in previously non-infested bodies of water, which may cause a loss in biodiversity and effect environmental health. The quagga mussel has spread rapidly from the eastern United States to the western United States since their discovery in Lake Erie in 1986. The quagga mussel was discovered in Lake Mead on January 6, 2007 at the Lake Mead Boat Harbor and Nevada has inherited the problems for which there are currently no known solutions. Lake Mead could contribute to the further spread of these dressenid species to non-infested bodies of water in the western United States, i.e. Lake Tahoe, due to

overland dispersal by contaminated watercraft. Previous studies on adult quagga mussels have been conducted on mussels east of the 100th meridian. The United States is host to multiple biomes that provide different climates for terrestrial and aquatic life to acclimate. To date there are no known studies on desiccation resistance with adult quagga mussels from the southwest region of the United States. The results of this study suggest that overland dispersal is possible depending on temperature and relative humidity. Based on this study, adult quagga mussels can survive for less than a day in hotter conditions (30°C or higher). In cooler conditions, adult quagga mussels can survive longer than five days. The data generated from this study may be helpful in preventing further establishment of the quagga mussel.

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Third, I would like to help all the individuals who have helped me along the way with my study. Dr. Paulo Pinheiro, for being a part of my thesis advisory committee even though his research interest is not in the field of quagga mussels. I want to let him know his time and advice was greatly appreciated. Dr. Steen Madsen for being my graduate college representative. Dr. Hae-sook Han for sitting down with me and providing me information with the tedious task of creating a stock solution of sodium hydroxide in order to control relative humidity. Thank you fellow graduate assistants within the Environmental Health Laboratory and UNLV School of Community Health Sciences for taking your time and assisting me in proofreading and providing support: Jamal

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CHAPTER 1

INTRODUCTION

The expansion of civilization across all borders of the world has proven to affect major components of ecosystems. If it were not for the European settlers who established the thirteen colonies along the Atlantic Coast of North America during the 17th century and the subsequent expansion of western civilization there would have been no development of international trade (initially shipping). Looking back at the arrival of European settlers in past centuries, they brought along complications and problems which caused the indigenous people to suffer. For example, early settlers introduced measles and smallpox to the Native Americans, ultimately decimating some native populations. In some cases entire tribes ceased to exist. This is an example of how a non-indigenous species can have an ecological impact on a new environment. Another example, in Hawaii, there was a huge problem with rats (introduced by western sailing ships). Since there were no natural predators such as snakes, the rat population exploded. In order to eliminate the rats, mongooses were introduced. Unfortunately, the rats were nocturnal and the mongooses were diurnal. The end result was that both species continued to expand their populations. As a result of expansion, there are many non-indigenous species that have been introduced to new environments and many indigenous species have become extinct due to the reduction of their natural environment and the decreases in natural resources needed to support their survival. This concept also applies to invasive species or nuisance species. The Invasive Species Advisory Committee (ISAC) created in January of 2000 by Executive Order 13112 defines an invasive species as “an alien

species whose introduction does or is likely to cause economic or environmental harm or harm to human health."

Much of our knowledge on aquatic invasive species (AIS) can be attributed to the study of ecology. The discipline of ecology addresses the environmental relationships in an ecosystem and studies the interactions between individual species that make up a community within that ecosystem. It is because of the study of ecology that we can better understand the environmental and economical impact each individual species has in an ecosystem between those interactions that take place within that community. The concept of an ecosystem is relatively simple; it involves those individuals that make the populations that create a community and their interactions among one another. The natural environment in an ecosystem includes those indigenous species that have established relationships with respect to one another (Molles, 2005).

It has been well documented that when a non-indigenous species is introduced into a foreign ecosystem that provides favorable conditions for its existence that the non-indigenous species creates ecological and economical impacts within that ecosystem (Pimentel et al., 2005; Leung et al., 2002; Lodge, 1993). In the United States, most cases of non-indigenous invasions in the aquatic environment have occurred with the unintentional dispersal through a contaminated vector such as a boat (Johnson et al., 2001; Griffiths et al., 1991) and via connected bodies of water (Johnson and Carlton, 1996). In the United States two AIS who have made a name for themselves in lakes and rivers are a pair of dreissenid mussels, native to Eastern Europe. Those two dreissenid mussels are known as *Dreissena polymorpha* and *Dreissena rostiformis bugensis* or more commonly known as the zebra mussel and quagga mussel, respectively. For the

remainder of this thesis the commonly used names zebra mussel and quagga mussel and their scientific names *D. polymorpha* and *D. bugensis*, respectively, will be used interchangeably. Since the discovery of dreissenid mussel species in Eastern Europe, and their subsequent relocation to the United States, they have found their way to as far West as San Justo Reservoir (San Benito, CA) and Lake Mathew (Corona, CA), respectively.

Lakes are able to support biological communities through what is known as the food web. Lake Mead's food web begins with the nutrients such as phosphorous and nitrogen, carbon dioxide and sunlight, which support phytoplankton. The phytoplankton supports the zooplankton and the shad. Shad are a type of herring found in Lake Mead and across rivers and lakes in the United States. Those zooplankton and shad support the game fish. The fact that the dreissenid mussel species, especially the quagga mussel, are profound filter feeders (Karatayev et al., 1997) serves as competition with the zooplankton and shad. It has been estimated that dreissenid mussel species can filter more than one liter of water per day (MacIsaac, 1996) and during reproduction a single female mussel can release thousands to millions of eggs (Mackie and Schloesser, 1996). While mortality rates of veligers (pre-adult mussels) may exceed 99% (Bially and MacIsaac, 2000) there are still enormous numbers of mussels that survive and are now found in Lake Mead. It has been estimated that there are over a trillion quagga mussels in Lake Mead (Cross et al., 2011). Their consumption of significant amounts of phytoplankton from the water has the potential to decrease the amount of zooplankton and shad, which may cause a disruption in the ecological balance of Lake Mead.

As a consequence of the introduction and spread of the dreissenid mussel species across the United States, many government agencies and States have commenced

working together to come up with solutions to deal with the devastating outcomes and potential problems created by dreissenid mussel species. The collaborative effort between government agencies and States has helped to develop statutes, policies and procedures and federal regulations in order to prevent and/or control the spread of mussels into uncontaminated bodies of water. Since the idea of spread has been thought of as overland dispersal by means of contaminated trailered boats (Cross et al., 2011; Britton et al., 2010; Johnson et al., 2001; Griffiths et al., 1991), many agencies have adopted boat inspection programs (Comeau et al., 2011). The 100th Meridian Initiative, created by the United States Fish and Wildlife Service, was designed to prevent the westward spread of AIS and to monitor and control AIS already established in bodies of water (Gerstenberger et al., 2004). The 100th Meridian (Figure 1) is defined by the 100th degree longitude that transects Texas, Oklahoma, Kansas, Nebraska, North Dakota and South Dakota (Gerstenberger et al., 2004).

Figure 1. 100th Meridian. Image from U.S. Fish & Wildlife Services. Retrieved [28 Aug 2012] from http://www.100thmeridian.org/Documents/100thMeridian.pdf

The 100th Meridian Initiative provides a tool to estimate the quarantine time a boat needs to remain out of water after being thoroughly cleaned. These quarantine times are based on evidence from laboratory experiments. Compared with other means of control (e.g chemical, pressure washing), desiccation has been proven to be 100% effective given an adequate amount of time. The main problems concerning desiccation are the environmental factors, temperature and humidity, and the amount of time needed to achieve 100% mortality. However, desiccation may not be convenient to the individual waiting for their boat. For example, the 100th Meridian Initiative quarantine estimates in the month of January it is recommended that the boat remain out of water for eight days (100th Meridian, 2011). In the month of August it is recommended the boat remains out of water for two days (100th Meridian, 2011).

To this date there are no known desiccation experiments conducted with quagga mussels west of the 100th Meridian. Desiccation studies have been conducted with dreissenid mussel species, primarily the zebra mussel, from the Great Lakes Regions and adjacent bodies of water east of the 100th Meridian's jurisdiction. In order to develop better approximate quarantine times it is important to consider mussels that had time to propagate and survive in a different climate. The recommendations of the 100th Meridian Initiative quarantine estimator are based in a report written by McMahon et al. (1993). There are other studies that present results that can be compared to McMahon et al. (1993). Ricciardi et al. (1995) conducted a laboratory experiment testing the aerial exposure of zebra mussels and quagga mussels by means of overland dispersal. Ricciardi et al. (1995) conducted their study with mussels in the St. Lawrence River southwest of the Island of Montreal. Now it is equally important to provide information regarding

aerial exposure in the arid southwest region of the United States. This information should evaluate combinations of temperature and humidity, which reflect different seasons of the year. It is of utmost importance to efficiently monitor and control the spread of dreissenid mussel species into uncontaminated bodies of water. The best control method for the spread of dreissenid mussel species is prevention.

Purpose of the Study

The primary purpose of this study was to determine the thresholds of aerial exposure that would result in 100% mortality among adult quagga mussels as a recommendation for future guidelines and protocols regarding decontamination and overland dispersal. This study was designed to provide valid experimental data on the amount of time adult quagga mussels can survive outside of their aquatic habitat at different combinations of temperature and relative humidity. Throughout the year temperatures fluctuate according to season; spring, summer, fall and winter. Climate fluctuates according to latitude and sea level and at any given time temperature and humidity fluctuate throughout the United States. For this study, the experiments were used to: (1) to establish the relationships between adult quagga mussels mortality by systematically testing different combinations of temperature and relative humidity in laboratory conditions, (2) determine the amount of time adult quagga mussels could survive outside of their aquatic habitat at different seasons of the year, if presented the opportunity to transfer from one body of water to another through some means of vector (such as a boat or other recreational equipment), and (3) validate those experimental

findings with results from fields studies done with encrusted boats pulled out of the water at Lake Mead National Recreation Area.

A second purpose of this study was that there are no known studies in the reviewed literature that have tested the aerial exposure of adult quagga mussels in the western United States. The United States presents multiple biomes and climates across the States (Figure 2). The difference in temperature and humidity across the States at different seasons of the year provides different opportunities for adult quagga mussels to remain viable outside of their aquatic habitat and become transferred through overland dispersal between different bodies of water.

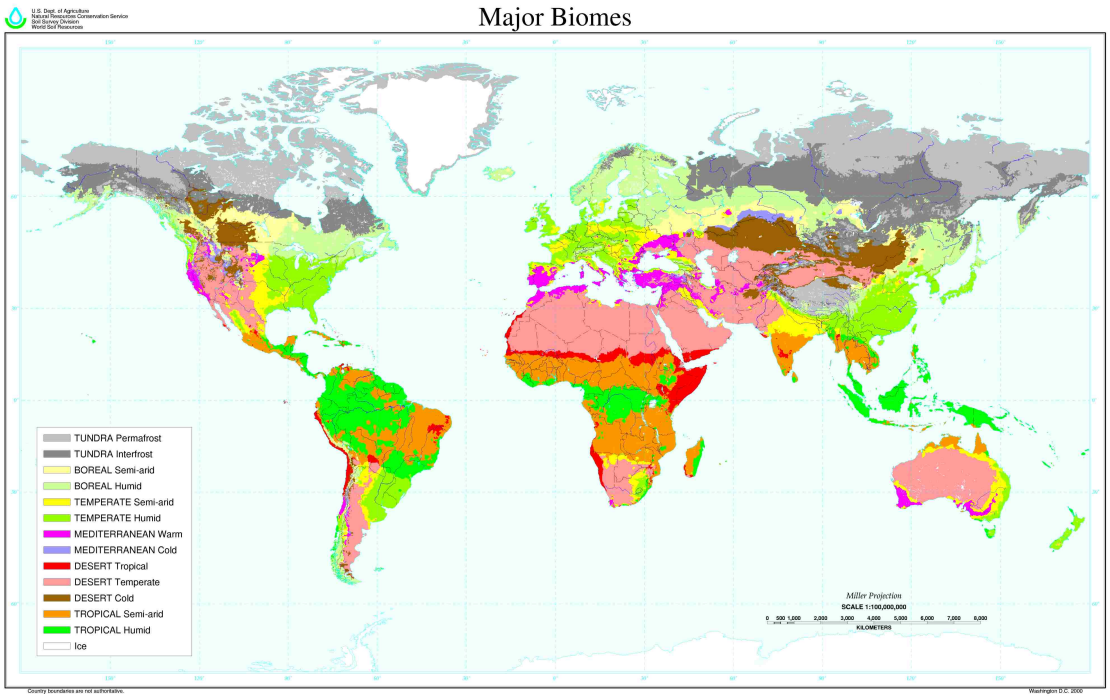

Figure 2. Major Biomes. Image from U.S. Dept of Agriculture. Retrieved [12 Jan 2012] from http://soils.usda.gov/use/worldsoils/mapindex/biomes.html

Research Questions

- How long can adult quagga mussels survive outside of their natural environment?
- At what temperature and humidity do they have a better chance of surviving for longer periods outside of their natural environment?
- At what time do adult quagga mussels reach 100% mortality between different combinations of temperature and humidity?
- How much variation is there between this study, previous studies and 100th Meridian Initiative's Drying Time Estimator?

Hypotheses

H_{A1}: There is a difference between the survivorship at different temperatures and relative humidity in adult quagga mussels

H_{A2}: The higher the relative humidity in he desiccating chamber, the greater the ability for adult quagga mussels to survive longer periods of aerial exposure

H_{A3}: The lower the temperature in the desiccating chamber, the greater the ability for adult quagga mussels to survive longer periods of aerial exposure

CHAPTER 2

LITERARY REVIEW

Origin and Spread

Since the introduction and establishment of dreissenid mussels in North America from their native environments in Eastern Europe they have become one of the most popular AIS studied. Within a decade they have been found as far west as California from their original discovery in the Laurentian Great Lakes. Since they are indigenous to water bodies across North America the fear of potential disruptions to indigenous ecosystems is mentioned with high interest in the reviewed literature. In 1890, Russian geologist, Nicolai Andrusov, discovered the quagga mussel in the Bug River, Ukraine. Andrusov later named the species in 1897 (Benson et al., 2011). Peter Pallas, a Russian naturalist, was the first to describe populations of zebra mussels in a tributary of the Ural River in 1897 (Benson and Raikow, 2011). Dreissenid mussels expanded from their native regions with the widespread construction of canals through Europe (Benson and Raikow, 2011; Benson et al., 2011; Mills et al., 1996). In 1988, the first dreissenid mussel, the zebra mussel, was discovered in Lake St. Claire between the Province of Ontario and the State of Michigan (Ricciardi et al., 1998; Hebert et al., 1989). The quagga mussel was discovered one year later, in 1989, near Port Colborne, Lake Erie (Mills et al., 1993). The popular theory behind their introduction into the North American waters is found in a multitude of reviewed literature. It is widely accepted that the dreissenid mussels were introduced into the Great Lakes through discharged bilge and ballast water from transoceanic ships from Europe (Ricciardi et al., 1998; McMahon, 1996; Carlton, 1993). Since their establishment in the Great Lakes both types of dreissenid mussels have

expanded across the United States (Figure 3). In January 2007, the quagga mussel was discovered in Lake Mead, NV-AZ (Mueting et al., 2010; LaBounty and Roefer, 2007). In May 2011, two reservoirs in Northern Nevada were found infested with the quagga mussel; Rye Patch Reservoir, south of Winnemuca and the Lahontan Reservoir, west of Fallon. Figure 4 shows the distribution of zebra mussels and quagga mussels in the western United States. The transport of trailered boats is a major suspect in overland dispersal of the mussels, first appearing in the Great Lakes Region then to the western United States (Nevada, Arizona, Colorado, Texas and Utah) (Cross et al., 2011; Britton et al., 2010; Johnson et al., 2001; Griffiths et al., 1991). The idea of overland dispersal through trailered boats as a means of introducing mussels to previously non-infested bodies of water has led many government agencies and national parks to adopt boat inspection requirements (Zook and Phillips, 2009).

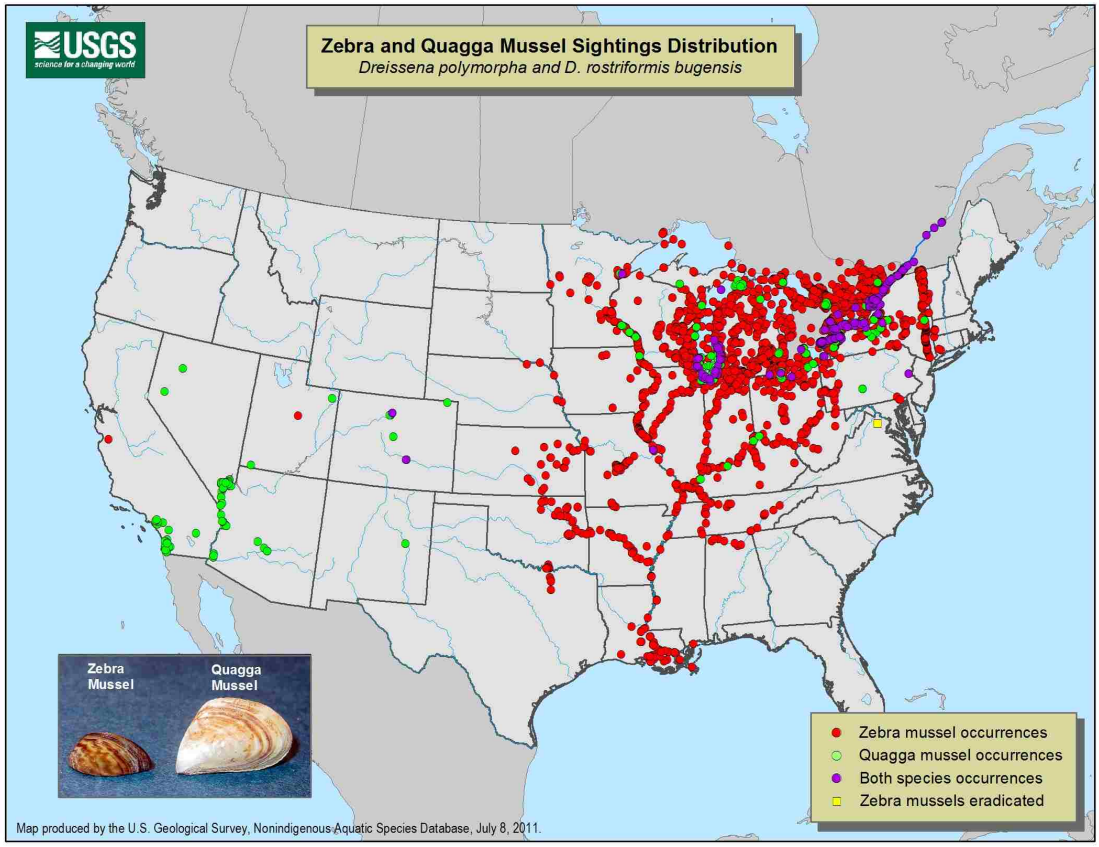

Figure 3. Zebra and Quagga Mussel Sightings Distribution. Image from USGS. Retrieved [15 Jan 2012] from http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/maps/current_zm_quag_map.jpg

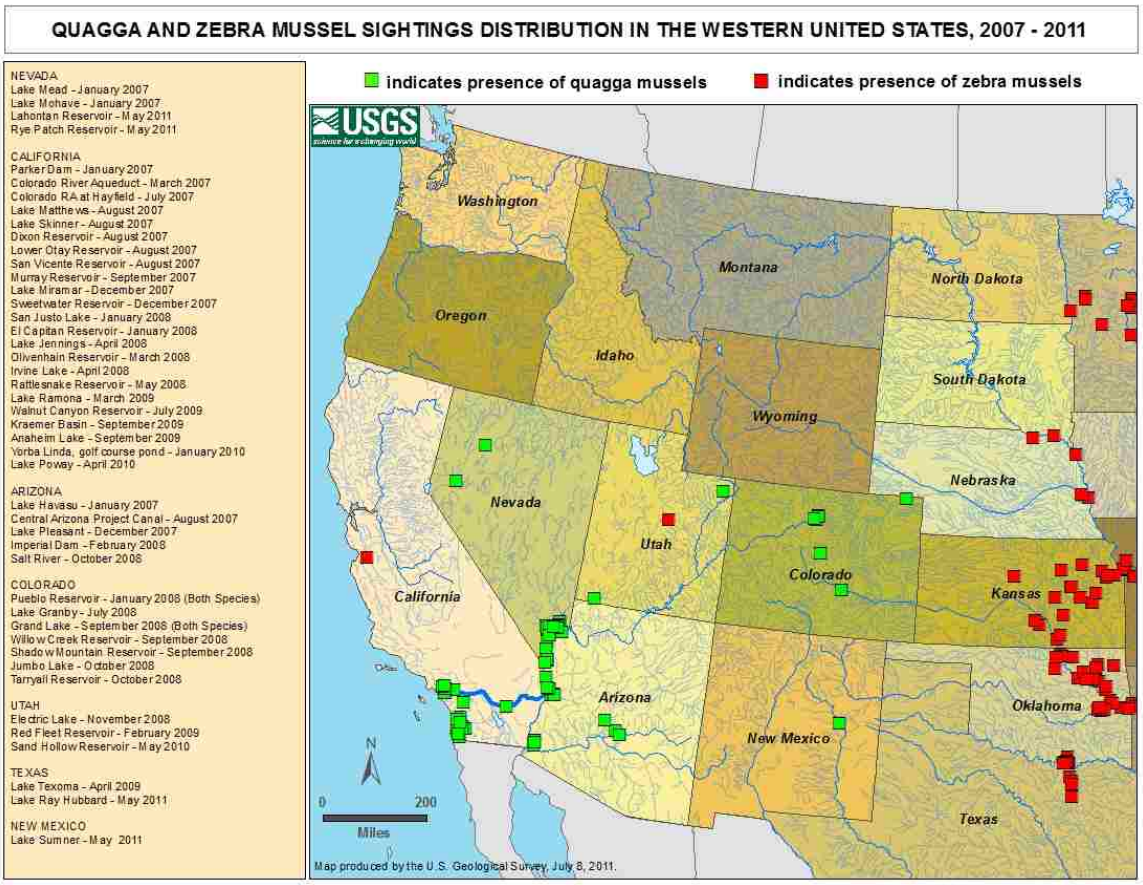

Figure 4. Quagga and Zebra Mussel Sightings Distribution in the western United States, 2007-2011. Retrieved [15 Jan 2012] from http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/maps/southwest_quagga.jpg

Ecological and Economical Impact

The introduction of non-indigenous aquatic species poses threats to the integrity and functioning of aquatic ecosystems and imposes huge economic costs to communities (Poulin et al., 2011). In recent decades, the focus of increasing study has been on the negative ecological impacts of aquatic invasive species (Holeck et al., 2004). Dreissenid mussel introduction can potentially have serious consequences to other components of the ecosystem including benthos, zooplankton, and phytoplankton (Caraco et al., 1997). The fact that dreissenid mussel species are filter feeders serves as competition with other

natural fauna. It has been estimated that dreissenid mussels can filter more than one liter of water per day (MacIsaac, 1996) and during reproduction a single female mussel can release thousands to millions of eggs (Mackie and Schloesser, 1996). While mortality rates of veligers (pre-adult mussels) may exceed 99% (Bially and MacIsaac, 2000) there are still enormous amounts of mussels that survive and continue to reproduce and create problems in lake ecosystems. It has been estimated that there are over a trillion quagga mussels in Lake Mead (Cross et al., 2011).

Throughout the reviewed literature the dreissenid mussel's ability to filter feed has been studied and discussed in detail. Their ability to filter feed can lead to changes and modifications in the food web. A public health concern of dreissenid mussels is the potential for bioaccumulation of chemicals hazardous to human health. Dreissenid mussels are reported to be candidates for bioaccumulations of hydrophobic contaminants such as polychlorinated biphenyls (PCBs), chorlinated insecticides (i.e. DDT) and polycylic aromatic hydrocarbons (PAHs) (Bruner et al., 1994). A major concern with controlling dreissenid mussels by means of chlorination is the by-product trihalomethanes, which are linked to adverse health effects and considered carcinogenic to animals (Watters, 2011; Cotruvo and Regelski, 1989). Along with alterations of food webs and bioaccumulation of contaminants is the ability to compete with indigenous species. In the reviewed literature, the deleterious effects of dreissenid mussels on indigenous North American freshwater mussel populations in the Laurentian Great Lakes have been well documented (Ricciardi et al., 1997; Gillis and Mackie, 1994; Schloesser and Nalepa 1994).

The introduction and establishment of dreissenid mussels has generated a great amount of focus on the ecological impacts. The economical impacts are consequences of those ecological impacts. In the United States, the annual cost and damages associated with AIS have been estimated to be over $7 billion (Pimentel et al., 2005). The fact that rivers offer ready access to water for recreational and industrial uses provides economical benefits to surrounding towns and cities. The Colorado River provides Southern Nevada with electricity, which is generated through Hoover Dam. Hoover Dam in turn, creates Lake Mead, a major recreational and fishing area. The Bureau of Reclamations has estimated that Hoover Dam's annual spending budget on quagga mussel control is $1 million a year (NDOW AIS Fact Sheet, 2011). Lake Mead is the major source of Southern Nevada's drinking water and accounts for more than 80% of the water used in the Las Vegas Valley (Mueting et al., 2010). According to the Southern Nevada Water Authority in 2011, they have spent $172,600 annually for chlorination additions, $34,000 for removal of quagga mussels from one drinking water intake tunnel, $6000 for routine maintenance and removal, $560,000 for proposed chemical control and $300,000 on research on the quagga mussel invasion (NDOWAIS Fact Sheet, 2011). The dreissenid mussel's ability to settle and colonize can clog water intake structures reducing the flow of water resulting in a constant monetary battle to prevent and control existing problems for the municipal water supply agencies, agricultural irrigation and power plant operations.

DREISSENID MUSSEL BIOLOGY

Lifecycle and Reproduction

The lifecycle of dreissenid mussels begins with an external process of reproduction (Ram et al., 1996). When water temperatures meet the optimal temperature, between 12 and 16º C (Claudi and Mackie, 1994), male and female gametes are released into the water beginning the process of fertilization. During spawning, a few individuals can produce millions of eggs and sperm (O'neill, 1993). Dreissenid mussels are generally found in clumps known as druses, which result in the close proximity between male and female mussels. This close proximity facilitates successful external fertilization (Ram et al., 1996). There are three main periods in the dreissenid mussel's lifecycle; the larval, juvenile, and adult stages (Crosier and Molloy, 2001). The first stage happens shortly after fertilization, within 6-96 hours, where a trocophore larva develops (Ackerman et al., 1994). It is during this stage that the velum is developed. The velum is the distinguishable feature that defines a veliger. The velum is the organ the trocophore larva uses for feeding and movement (Ackerman et al., 1994). The second stage of the mussel lifecycle occurs between two to nine days post fertilization. At this time the shell glands secrete a D-shaped or straight-hinged shell (Crosier and Molloy, 2001). The third stage is the final free-floating stage and is referred to the umbonal veliger, also known as the veliconcha where the shell has become more pronounced and round in shape (Mackie, 2004; Claudie and Mackie, 1994). After ten days post fertilization the umbonal veliger develops into the pediveliger where many anatomical features develop such as the foot, siphon and byssal apparatus (Mackie and Claudi, 2009; Ackerman et al., 1994). Settlement and byssal attachment to substrates begins during the pediveliger stage (Mackie, 2004) and the

pediveliger eventually becomes a juvenile mussel (Ackerman et al., 1994). Once juveniles become settled and attached to a substrate they grow up to 23 mm (zebra mussels) and 38 mm (quagga mussels) (Mills et al., 1996) until they reach sexual maturity, to spawn and start the lifecycle over again.

Anatomy and Morphological Differences

Dreissenid mussels are freshwater bivalve mollusks that are in the class Bivalvia (McMahon, 1991). These mussels have an elastic hinge ligament that connects the two shells (McMahon, 1991). The elasticity of the hinge ligament functions to separate and open the mussel when the adductor muscles are relaxed (Mackie and Claudi, 2009). Dreissenid mussels have two shell adductor muscles, the anterior and the posterior, which run between the valves and function to close the valves upon contraction (Mackie and Claudie, 2009). When dreissenid mussels die, their shells will gape or spread apart (Ricciardi et al., 1995). The mantle is the thin tissue that secretes the shell and covers the mussel's major internal organs (Mackie and Claudi, 2009). The mantle helps protect organs from sediment abrasion and contamination (McMahon, 1991). The mantle forms two openings: the inhalant and exhalant siphons (Claudi and Mackie, 1994). Located on the ventral surface of the shell is an opening in the mantle known as the pedal gap that allows for the extrusion of the foot and byssus for locomotion and settlement, respectively (Mackie and Claudi, 2009; Morton, 1993).

Since both zebra mussels and quagga mussels are from the same genus Dreissena their reproduction, lifecycle and feeding habits are similar but they are still two different species. The most noticeable distinguishable features between zebra mussels and quagga

mussels are the morphological differences in size and shell appearance. If both species of dreissenid mussels are placed on a flat surface such as a countertop the zebra mussel will remain upright (Claudie and Mackie, 1994) because of a definitive angle between that ventral and dorsal surfaces (May and Marsden, 1992) that gives the zebra mussel a flat appearance. The complete opposite occurs with the quagga mussel when placed on a flat surface. The quagga mussel will topple over (Claudie and Mackie, 1994) because the quagga mussel has a convex ventral side (May and Marsden, 1992) that gives the quagga mussel a rounder appearance. Another morphological difference between zebra mussels and quagga mussels can be seen when viewing the mussels from either the front or the ventral side. The zebra mussel has bilaterally symmetrical shells joined together and forming a straight midventral line (Figure 5), whereas, the quagga mussel's shells are asymmetrical and there is not a straight midventral line (Figure 5) (Domm et al., 1993). Zebra mussels are smaller in size compared to their cousin the quagga mussel. The zebra mussel reaches up to 23 mm, whereas, the quagga mussel reaches up to 38 mm (Mills et al., 1996).

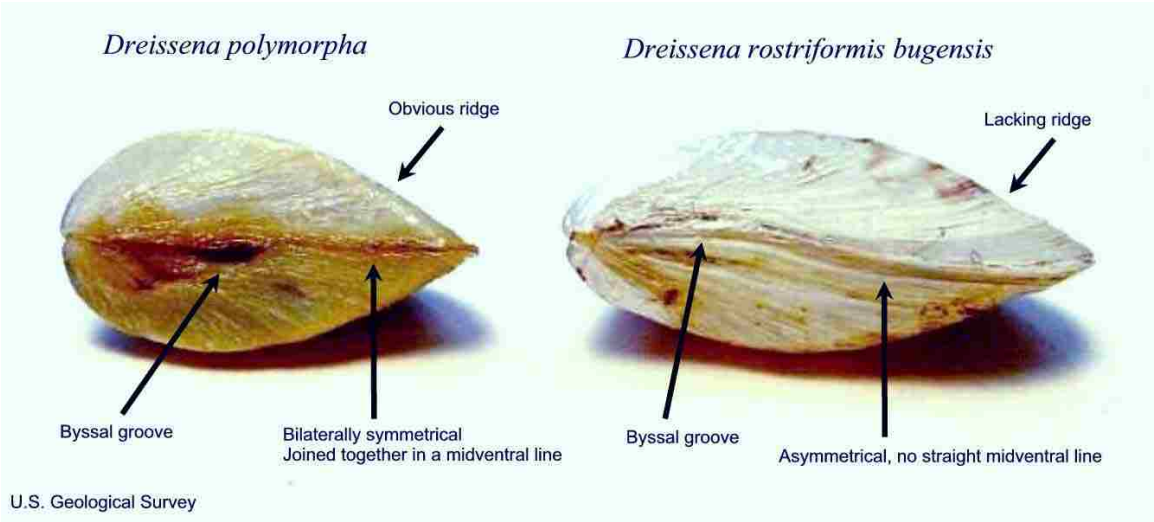

Figure 5. Morphological Differences of Dreissenid Mussels. Image from USGS. Retrieved [17 Jan 2012] from

http://fl.biology.gov/Nonindigenous_Species/Zebra_mussel_FAQs/Dreissena_FAQs/zebra_quagga_ventral.jpg

Substrate Settlement

Settlement and byssal attachment occurs when dreissenid mussel colonies find a suitable substrate during their pediveliger stage (Mackie, 2004). Settlement is the active process when pediveligers swim or crawl searching for a suitable firm substrate (Ackerman et al., 1994). Although hard, calcareous materials are preferred substrates for dreissenid mussels, they will attach to various surfaces (Benson et al., 2011). They have been found to successfully colonize rocks, limestone, concrete, boat hulls, boat motors, docks, buoys, aluminum, water intake valves and pipes, and vegetation (Watters, 2011; Benson et al., 2011; Mueting et al., 2010).

Methods of Control

Since the introduction and establishment of dreissenid mussels in the United States, a major focus of scientists and government agencies has been to control the number of mussels in contaminated bodies of water and prevent the spread of dreissenid mussels into uncontaminated bodies of water. The methods used to control dreissenid mussels must consider potential adverse affects on the surrounding environment. Although some methods of control are more effective than others when preventing further spread of dreissenid mussels and controlling established populations those methods may not always be feasible, so generally the safest route is chosen. Some of the dreissenid mussel methods of control include the following; physical and mechanical cleaning, chemical control, thermal control, pressurized hot-water spray, freezing and

dessication. Since the early 1990s the U.S. Army Corps of Engineers has performed several studies on both the zebra mussel and quagga mussel. Those studies range from environmental testing, control methods and control strategies. Studies in the reviewed literature, however, are not limited to the U.S. Army Corps of Engineers. For this thesis, the following will provide a brief overview of the methods of control found in the reviewed literature along with how those methods have been applied to decontamination of watercraft and equipment as a means of prevention of overland dispersal.

Physical and Mechanical Cleaning

As a process of pre-cleaning dreissenid mussels can be removed from infested watercraft or equipment by hand, brush, scrapers or other physical means. This is an effective method of control for adult dreissenid mussels but must be repeated regularly and the manual labor is not as cost effective as other preventative methods of control (Boelman et al., 1996). Physical and Mechanical cleaning should also be done as a method of pre-cleaning and another form of decontamination should be performed to ensure 100% effectiveness (USBR, 2010). This form of cleaning is not effective against the removal of planktonic veliger stages of dreissenid mussels due to their size being undetectable by the human eye. The disadvantage of physical and mechanical cleaning is the amount of time taken to remove adult mussels from watercraft especially those with large surface areas and the equipment on the watercraft that is not easily accessible.

Chemical Control

Chemical control is one of the more widely used methods of control in the United States (Claudi and Mackie, 1994). This method of control can be considered effective but must take into consideration the surrounding environment, the health of individuals and

must be in compliance with environmental regulations (Sprecher and Getsinger, 2000). The application of chemicals is based on the lifecycle of the dreissenid mussel, the chemical applied, and the amount of time needed to ensure 100% mortality (USBR, 2010). Claudi and Mackie (1994) proposed five different treatment strategies; end-of-season, periodic, intermittent, continuous, and semi-continuous. One of the most popular and effective means of chemical control is chlorination (Claudi and Mackie, 1994). However, chlorination has a by-product called trihalomethane and is considered to have adverse health effects on humans and can be carcinogenic in animals (Watters 2011; Cotruvo and Regelski, 1989). Effective treatments include both oxidizing chemicals (chlorine, potassium, and hydrogen peroxide) and non-oxidizing chemicals (molluscicides, copper sulfate, and metal ions) (Beyer et al., 2011). As previously stated, concerns arise about the use and disposal of strong oxidizing chemicals and potentially toxic chemicals (Beyer et al., 2011).

The United States Bureau of Reclamation's "Inspection and cleaning manual for equipment and vehicles to prevent the spread of invasive species" suggests a few effective methods for decontamination using chemicals once equipment and watercraft have been pulled from infested water. The following suggestions for equipment and watercraft decontamination are diluted household bleach applied to the infested watercraft for a minimum of an hour, undiluted vinegar for 20 minutes, a 1% potassium permanganate solution for 24 hours, and a 5% quaternary ammonium solution for 10 minutes (USBR, 2010).

Thermal Control

Thermal treatment of raw water systems is generally an accepted non-chemical method of treatment in the control of dreissend mussels (McMahon and Ussery, 1995). Thermal treatment is based on two approaches, the acute lethal temperature or the chronic upper lethal temperature (McMahon and Ussery, 1995). Acute upper lethal temperatures are defined as the temperature at which death occurs when water temperature is raised during the treatment. Chronic upper lethal temperatures are defined as the continuous exposure to a constant lethal temperature for a period of time to induce mortality. Thermal control is an effective way of killing dreissenid mussels but depends on the temperature and exposure times to determine 100% mortality. There is a plethora of literature on the effects of thermal treatment on dreissenid mussels (i.e. Beyer et al., 2011; Elderkin and Klerks, 2005; Rajapogal et al., 2005; McMahon, 1996; Armistead, 1995; McMahon and Ussery, 1995; Spidle et al., 1995; McMahon et al., 1993; Iwanyzki and McCauley, 1992; Jenner and Jansen-Mommen, 1992).

Quagga mussels are less tolerant than zebra mussels when exposed to warmer water temperatures (Spidle et al., 1995). This is believed to be due to the fact that quagga mussels have thinner shells (Zhulidov et al., 2006) that seal less tightly (Claxton et al., 1997) than their counterpart, the zebra mussel (Comeau et al., 2011). Depending on acclimation temperatures the upper incipient lethal temperature for zebra mussels is 31º C and they will not survive for prolonged periods of time (McMahon and Ussery, 1995). Spidle et al. (1995) found that quagga mussels could survive no longer than 14 days (100% mortality) in water temperatures of 30º C. When both species of mussels were tested at 35º C, 100% mortality was achieved within 24 hours (Spidle et al., 1995). Of the

studies in the reviewed literature, the results have shown that the relationship between temperature and exposure time is that as the temperature is increased the amount of exposure time to reach 100% mortality is decreased (i.e. Beyer et al., 2011; Spidle et al., 1995). Thermal treatment studies have served as a background for hot-water spray as a means of boat and equipment decontamination for prevention of overland dispersal into uncontaminated bodies of water. Temperature and exposure time determine the effectiveness of decontamination and control (USBR, 2010).

Pressurized Hot-Water Spray

Pressurized hot water washing of contaminated watercraft surfaces is the most widely accepted method of decontamination. What makes this method of decontamination more effective is the combined use of a high-powered pressure water (3,000 psi) and heat (no lower than 60º C) (USBR, 2010). Morse (2009) tested zebra mussels by means of hot-water spray to access the effects of application time and temperature. The results found that survivorship decreased with both increased exposure time and increased test temperatures (Morse, 2009). Zebra mussels sprayed at 20º C exhibited 100% survivorship in the study. Survival rates were high in samples exposed to 1-second spray duration at temperatures ≤ 60º C and spray exposures of 5 seconds or 10 seconds did not induce 100% mortality at temperatures ≤ 50º C (Morse, 2009). Spray exposures of 5 seconds at 80º C and 10 seconds at 60º C resulted in 100% mortality (Morse, 2009). Comeau et al. (2011) conducted a similar study with the use of hot-water spray as a means of watercraft decontamination with quagga mussels in Lake Mead, NV. Results agreed with Morse (2009), the relationship between high temperatures and longer

exposures induce greater mortality. 5-second spray exposures of ≥ 60º C resulted in 100% mortality (Comeau et al., 2011).

The United States Bureau of Reclamation's "Inspection and cleaning manual for equipment and vehicles to prevent the spread of invasive species" suggests a few effective methods for decontamination by means of hot-water spray. Those suggestions are as follows:

- Use a 3,000 psi pressure washer that is capable of applying a flow rate of at least 4 gallons/minute and able to supply water ≥60º C (USBR, 2010).
- Leave the attached mussels on the surface because the goal is to kill adult mussels with hot water while they remained attached to the surface (USBR, 2010).
- Rinse the entire surface for a minimum of 30 seconds at 60º C at a reduced pressure (USBR, 2010).
- After rinsing for a minimum of 30 seconds at 60 º C, maintain a hot-water temperature and increase the pressure to detach dreissenid mussels from the surface (USBR, 2010).
- Continue treatment on all exposed surfaces of the equipment and use a flushing attachment for hard to reach areas or areas where high pressure may damage equipment. For those areas where high pressure may damage equipment maintain a hot-water contact time of 2-3 minutes (USBR, 2010).

Freezing

Subsequent freezing of dreissenid mussels during the winter can be effective to those mussels found exposed to freezing temperatures (Boelman et al., 1996). Payne (1992a) conducted a series of laboratory experiments to test the survival of aerially

exposed zebra mussels, separated and clustered, to air temperatures ranging from 0º to -10º C. The results found that for separate individual zebra mussels, the time required for 100% mortality ranged from approximately half hour at -10º C and longer than 48 hours for 0º C (Payne, 1992a). For clustered zebra mussels, the time required for 100% mortality ranged from approximately 2 hours at -10º C and longer than 48 hours at both -1.5º C and 0º C (Payne, 1992a). In a similar study conducted by McMahon et al. (1993) mussels were found to be greatly tolerant of freezing temperatures. Their results found that separate individual zebra mussels achieved 100% morality in all treatments except 0º C within 48 hours of testing (McMahon et al., 1993). For clustered zebra mussels, tolerance of freezing temperatures increased at all test temperatures (McMahon et al., 1993). There are no known freeze studies conducted on quagga mussels in the reviewed literature.

Desiccation

Desiccation is the process of dehydration resulting from the removal of water. This means of control provides another non-chemical approach to control the spread of dreissenid mussels and can be 100% effective determined by the time dreissenid mussels are exposed to air, outside of water, and the temperature and relative humidity in which they desiccate. MacMahon et al. (1993) conducted a series of laboratory experiments to assess the time required to reach 100% mortality in adult zebra mussels treated to a combination of five relative humidities and three temperatures. The results of that study found that the survival time of adult zebra mussels aerially exposed was greatly increased by higher humidities at lower temperatures (McMahon et al., 1993). Ricciardi et al. (1995) conducted a similar study with zebra mussels and quagga mussels with a

combination of exposure treatments (temperature and relative humidity). Their study comprised of two zebra mussel lengths; 10.0-18.0 mm and 21.0-28.0 mm. Quagga mussels were based on one mussel size due to the limited number in the study. Ricciardi et al. (1995) found that zebra mussels reach 100% mortality after 5 days when exposed to 20º C at 10% relative humidity, after 3 days when exposed to 30º C at 10 and 50% relative humidity, and after 5 days when exposed to 30º C at 95% relative humidity regardless of the size of the mussel. The quagga mussels in the study achieved 100% mortality after 3 days when exposed to 20º C and 10% relative humidity, after 3 days when exposed to 20º C at 50% relative humidity, and after 5 days when exposed to 20º C at 95% relative humidity. Fewer trials were run with the quagga mussels because of the limited numbers collected in the study.

Desiccation is an effective method of control if given sufficient time depending on the climate; month, location and relative humidity. It has been estimated that dreissenid mussels can survive for over 40 days outside of water in cooler, extremely humid areas (USBR, 2010). The 100th Meridian Initiative established a quarantine estimator for drying contaminated boats which can be found on their website (http://www.100thmeridian.org/emersion.asp). The recommendations for the quarantine estimator are based on a U.S. Army Corps of Engineers Contract Report written by McMahon et al. (1993).

Environmental Policy

Since 1990 many States have adopted statutes, policies and procedures, and implemented federal regulations to control and prevent the spread of dreissenid mussels. Those governmental agencies involved with dreissenid mussels are the Environmental

Protection Agency, the Department of Wildlife, Bureau of Reclamations, Department of Fish and Game, U.S. Fish & Wildlife Service, United States Department of Agriculture, along with many local businesses and universities (ANS Task Force, 2012). Together those agencies, businesses and institutions spend great amounts of money to maintain the quality of water, to protect natural ecosystems, to contain existing infestations, and to prevent further spread of dreissenid mussels. Some of the major policies passed through the legislature are the Non-indigenous Aquatic Nuisance Species Prevention and Control Act (NANPCA) of 1990, the National Invasive Species Act of 1996, Executive Order 13112 (February 3, 1999), and the Clean Boating Act of 2008. As a result of the NANPCA of 1990, the Aquatic Nuisance Species (ANS) task force was created. This task force is composed of representatives from the U.S. department of the Interior, U.S. Fish and Wildlife Services, Environmental Protection Agency, U.S. Department of Defense, Army Corps of Engineers and the U.S. Department of commerce, and the National Oceanic and Atmospheric Administration (ANS Task Force, 2012). The responsibility of the ANS task force is to identify areas of environmental threat, assess the ecological and economical characteristics threatened, determine the need for methods of control on watercraft, and evaluate the risk and consequences associated with invasive species which are found under the provisions of the NANPCA of 1990 (ANS Task Force, 2012). Two major public awareness campaigns are the Stop Aquatic Hitchhikers and the 100th Meridian Initiative.

Stop Aquatic Hitchhikers

The Stop Aquatic Hitchhikers is a public awareness campaign created by the ANS task force. The campaign is designed to educate recreational boaters to help prevent the

spread of ANS. Stop Aquatic Hitchhikers calls for those individuals who use lakes for recreational purposes to be part of the solution rather than part of the problem. The following actions are asked of recreational boaters (Stop Aquatic Hitchhikers, 2011):

- Understand the basic problems and solutions
- Follow the recommended procedures for cleaning items used in the water
- Avoid releasing fish/animals/plants into waters
- Help inform others
- Get involved in policy and legislative solutions

The 100th Meridian Initiative

The 100th Meridian Initiative is a cooperative effort between local, state, provincial, regional and federal agencies to prevent the westward spread of dreissenid mussels and other ANS in North America. The goals of the 100th Meridian Initiative are to prevent, monitor and control ANS in established bodies of water within the 100th Meridian's jurisdictions. The 100th Meridian Initiative developed seven components to achieve their goals: 1) information and education, 2) voluntary boat inspections and boater surveys, 3) involve those who haul boats for commercial purposes, 4) monitoring, 5) rapid response, 6) identify pathways and risk evaluation, and 7) evaluation (100th Meridian Initiative, 2011).

CHAPTER 3

METHODOLOGY

Desiccation of Adult Quagga Mussels

Desiccation is an accepted approach by the Aquatic Nuisance Task Force as a means of boat and equipment decontamination to prevent overland dispersal of ANS. The 100th Meridian Initiative which is a cooperative effort to prevent the westward spread of ANS developed a boat quarantine estimator which recommends time (in days) that a boat should remain out of water depending on the location, temperature and relative humidity. In the reviewed literature, studies using desiccation in a laboratory setting date back to the early 1990's and a majority of the information regarding desiccation in the literature is with zebra mussels. The 100th Meridian Initiative's Boat Quarantine Estimator is based on a study conducted by McMahon et al. (1993). Data from this study suggests mussels need at least 3 days aerial exposure to achieve 100% mortality at 25°C (77°F) and low relative humidity. Temperatures below 15°C (59°F) with high humidity may be greater than 10 days to 100% mortality (McMahon et al., 1993). Payne (1992b) wrote a U.S. Army Corps of Engineers technical report based on the previous studies conducted by McMahon et al. (1993). Payne (1992b) wrote in his report that aerial exposure should last nearly a month when zebra mussels are exposed to temperatures as low as 5°C (°F) and humidity as high 95%. When exposed to the opposite conditions, 25°C (77°F) and 5% relative humidity the time needed to achieve 100% mortality was only two days (Payne, 1992b). A second study conducted by Ricciardi et al. (1995) provided results from different combinations of treatment with both zebra mussels and quagga mussels. A limitation of this study was the small amount of quagga mussels collected. Because of the

abundance of zebra mussels in the study they were exposed to combinations of three different temperatures (10, 20 and 30°C) and three different relative humidities (10, 50 and 95%) with three different exposure durations (1, 3 and 5 days); whereas, quagga mussels were subjected to laboratory conditions of 20°C at the three different relative humidities and exposure durations. The previous studies provide useful data for protocols that can be utilized for quarantine such as the quarantine estimator provided by the 100th Meridian Initiative. Data from previous studies suggests an inverse relationship between mortality and exposure to temperature and humidity (Ricciardi et al., 1995; McMahon et al., 1993). The higher temperatures at lower humidity require less time to achieve mortality compared to lower temperatures at higher humidity, which require more time to achieve the desired outcome.

Most of the reviewed literature on zebra mussels and quagga mussels took place east of the 100th Meridian. The reason being both mussels were first discovered in the Laurentian Great Lakes. Unfortunately, those mussels have found their way as far west as California. The United States is host to multiple biomes that provide different climates for terrestrial and aquatic life. Temperature and humidity fluctuates between each biome. Studies in the reviewed literatures have mentioned that tolerance of dreissenid mussels depends on the temperatures in which they were acclimated (Beyer et al., 2011; McMahon and Ussery, 1995; Ricciardi et al., 1995). The reviewed literature also references the difference between zebra and quagga mussel tolerance to temperature treatment (Mills et al., 1996; Comeau et al., 2011). Quagga mussels have thinner, less tightly sealed shells compared to their counterpart, the zebra mussel (Zhulidov et al., 2006; Claxton et al., 1997). That may be the reason for differences found in upper

thermal limit. In the reviewed literature, quagga mussels were shown to have a lower upper thermal tolerance compared to zebra mussels (Mills et al., 1996). Therefore, quagga mussels may not be able to retain as much water as the zebra mussel when subjected to desiccation.

A study needs to be performed on quagga mussels in the western United States by means of desiccation because the quagga mussel is the most invasive and dangerous mussel in the western United States (Wong and Gerstenberger, 2011). Most studies have tested zebra mussels in the Laurentian Great Lakes. This study investigates a wide spectrum of combinations of temperature and relative humidity to determine the amount of time to achieve 100% mortality for adult quagga mussels at each combination. The combinations of exposure will allow adult quagga mussels to experience laboratory climates experienced in the western United States. Adult quagga mussels will experience temperature ranges from 10 to 40º C, relative humidity ranging from 20 to 80% for an exposure duration of 1, 3 and 5 days. The data from this study was compared to previous studies on dreissenid mussels to determine if there is any difference between quagga/zebra mussels from Laurentian Great Lakes and quagga mussels from Lake Mead. The data from this study can be used as a consideration along with previous studies for quarantine time of watercraft and equipment.

Specimen Collection and Holding Conditions

Adult quagga mussel specimens (≥ 14 mm in length) were carefully collected along the boat docks at Hemenway Harbor, Lake Mead, NV (GPS: 36º 01’48.01” N, 114º 46’13.74” W) by hand. Those mussels were then transported from the dock in a 5-gallon

plastic bucket filled with lake water to the Nevada Department of Wildlife's (NDOW) Fish Hatchery where they were placed into multiple 10-gallon flow-through aquariums. The mussels were kept in the flow-through tanks and were fed Instant Algae® *Isochrysis* (Reed Mariculture, Inc) periodically until experimentation. The acclimation process was no less than seven days. The acclimation water temperature in the holding tanks at the NDOW's Fish Hatchery was 15°C ± 2.

Experimental Design and Measurements

After acclimation, adult quagga mussels were separated by size into druses of ten individuals each. Druses of ten mussels were achieved by carefully cutting the byssal threads of excess mussels with small scissors, trying to present the least amount of stress to surrounding mussels. Mussels that appeared to be smaller than 14 mm were carefully cut away from the druse. Prior to experimentation, desiccating chambers were created by using 1000 mL Pyrex beakers (VWR International Inc.) filled with aqueous sodium hydroxide solutions to maintain constant relative humidity as determined by Madge (1961). Mussels were placed on a mesh support above plastic supporters a few centimeters above the solution in the desiccating chamber to prevent contact with the aqueous solution (Ricciardi et al. 1995).

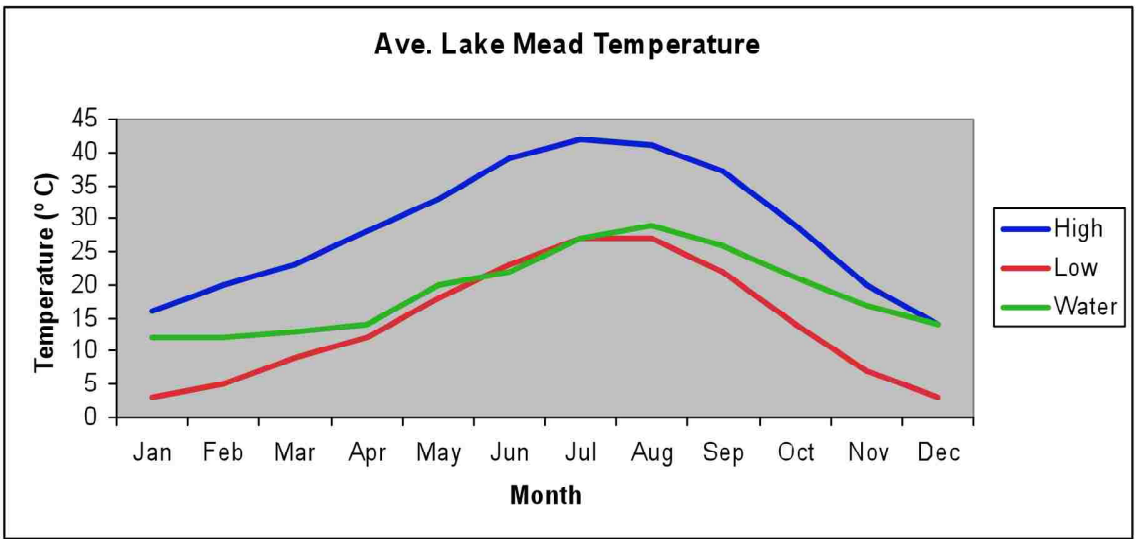

Figure 6. Average Maximum and Minumum Air Temperatures Lake Mead, NV. Data from http://www.houseboating.org/MEAD/temperatures.cfm.

The mussels were separated and placed onto a mesh net in the desiccating chambers and then treated to different combinations of temperature and relative humidity for an exposure period of 1, 3 and 5 days. The experimental design is similar to the study design used by Ricciardi et al. (1995). The combination of treatment and relative humidity was determined to present a wide range of the climate spectrum at Lake Mead, NV during different seasons; dry and moist, hot and cold. Figure 6 shows the average maximum and minimum air temperatures experienced through the year at Lake Mead, NV. Two identical REVCO™ Ultima II Laboratory CO_2 Incubators were used to constantly maintain temperatures of 30 and 40˚C, respectively. A 3.2 cu. ft. GE Compact Refrigerator (Model: SFR03GAZBB) was used to constantly maintain 10˚C while 20˚C was achieved by leaving the desiccating chambers on a laboratory table at room temperature. The beakers were sealed with plastic wrap and rubber bands to create a closed environment. The relative humidity of each desiccating chamber was measured daily by inserting a digital thermometer/hygrometer (GENERAL® Model: PTH8708)

inside of the beaker. This procedure was done quickly to prevent as much environmental exchange (internal and external) as possible. Oxygen was replenished in each chamber each day when the relative humidity was measured in the desiccating chamber. Samples of mussels were then treated to combinations of temperatures at 10, 20, 30 and 40° C, relative humidity of 20, 50 and 80% and exposure durations of 1, 3 and 5 days. Average daily temperature and relative humidity fluctuations were ± 0.96 and ± 9%, respectively. Each treatment sample was replicated four times. A total of 36 treatment combinations were conducted in this experiment with a total of 1,440 adult quagga mussels (40 mussels per treatment combination) (Table 1). Upon the completion of each treatment, the mussels will then be taken back to the flow-through tanks at the NDOW Fish Hatchery and given a recovery period for not less than two days. The control group for this experiment was 40 mussels per exposure duration (1, 3 and 5 days) kept in treatment tanks (10, 20, 30 and 40° C) that had de-chlorinated, plankton free tap water that was continuously aerated. Tap water was de-chlorinated with API™ Tap Water Conditioner (Mars Fishcare Inc.).

Table 1. Study Design: Desiccation of Adult Quagga Mussels

Temperature		Relative Humidity 20%			50%			80%		
ºC	ºF	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5
10	50	40*	40*	40*	40*	40*	40*	40*	40*	40*
20	68	40*	40*	40*	40*	40*	40*	40*	40*	40*
30	86	40*	40*	40*	40*	40*	40*	40*	40*	40*
40	104	40*	40*	40*	40*	40*	40*	40*	40*	40*

N = 1440 adult quagga mussels, shell length > 10 mm
n = 40 adult quagga mussels per treatment, shell length > 10 mm
*4 druses of 10 mussels

Data Collection and Management

After the recovery period, mussels were tested for viability according to methods established by McMahon and Ussery (1995) and Ricciardi et al. (1995) and measured for size with digital calipers (Model 62379-531; VWR International, Inc). Mortality is assessed when an extended gape in the dreissenid mussel's shell valve is observed. If mussels express an extended gape they will still be tested for viability. Viability of adult quagga mussels was determined after a 24 hours period by gently touching the tissues of the posterior mantle edge or siphons with a prodding scalpel to stimulate mussel response. If adult quagga mussels did not respond to the prodding by shell valve closure, the mussels were considered to be dead. The shell length of each adult quagga mussel was the distance measured from the posterior edge of the shell to the anterior tip of the umbos (McMahon and Ussery, 2005). The shell length was measured to the nearest 0.1 mm with a digital caliper (Model 62379-531; VWR International, Inc). All data was recorded in a laboratory notebook and converted into a Microsoft Excel sheet for use with statistical analysis software and stored on an external SanDisk Cruzer flash drive.

Statistical Analyses

A 3-way analysis of variance (ANOVA) was performed on the independent variables temperature, relative humidity and exposure duration (days) with the dependent variable, mortality, for adult quagga mussels in the desiccation experiment followed by analysis with the Student-Newman-Keuls (SNK) post-hoc test. The same data was run through a multiple regression model to learn more about the relationship between temperature, relative humidity and exposure duration to mortality. Both statistical

analyses were also performed with percent weight loss. Analysis of covariance (ANCOVA) was conducted with the validation experiment results (spring and summer field validation experiments) to compare the mortality rate at different seasons (spring vs summer).

The significance criterion used in this study was $\alpha = 0.05$. All statistical analysis and model estimation were performed with SAS® (Version 9.2, SAS Institute Inc. Cary, NC).

Summer and Spring Boat Validation

For both summer (August 2011) and spring (March 2012), a mussel-encrusted boat was voluntarily obtained from the Lake Mead Marina (GPS: 36º 01'48.01" N, 114º 46'13.74" W) and a second boat from Cottonwood Cove, Lake Mohave, NV (GPS: 35º 29'34.49" N, 114º 41'04.40" W), respectively. Adult quagga mussels were carefully collected from the encrusted boats by hand at certain time periods and transferred to the Lake Mead Fish Hatchery in a 5-gallon bucket filled with lake water. Summer boat collections were collected at time(s) 0, 12, 24, 48, 72, 120 and 168 hours. Spring boat collections were collected at time(s) 0, 0.5, 1, 2, 4, 6, 12, 48 and 72 hours. The temperature of the water was measured at time 0. The temperature and relative humidity was recorded daily. The boat was divided into four sections and twenty mussels were collected from each section at each time and placed in a pre-labeled 3.0 mm mesh spat bag (Aquatic Eco-Systems Inc, Apopka, FL). The mussels were placed in a bucket filled with Lake Mead water and placed into the flow-through tanks at the Lake Mead fish hatchery where they were tested for viability after a 24-hour recovery period. The control

group consisted of the adult quagga mussels collected at time 0. For the spring boat validation experiment mussels were collected in labeled spat bags and placed back into Lake Mohave until they were ready to be transported back to the NDOW fish hatchery flow-through tanks. Mussels were transported from Cottonwood Cove, Lake Mohave, NV to NDOW fish hatchery Lake Mead, NV into a 10-gallon insulated Styrofoam cooler filled with Lake Mohave water.

CHAPTER 4

FINDINGS OF THE STUDY

Mortality of *D. bugensis* exposed to desiccation

3-way ANOVA and SNK post-hoc test

The variables temperature, relative humidity and exposure duration explained 98% of the observed variance in mortality among druses of *D. bugensis* (ANOVA, $F_{35, 108} = 142.78$, $p < 0.0001$, $r^2 = 0.978$). The three-way ANOVA found that all two-way interactions and the three-way interaction between temperature, relative humidity and exposure duration with mortality were significant (Table 2). The SNK post-hoc analysis for morality and relative humidity showed that relative humidity (RH) was significantly different with the highest mortality occurring at 20% RH and the lowest morality occurring at 80% RH. The SNK post-hoc analysis for mortality and temperature showed that there was no significant difference in 30 and 40º C. The least amount of mortality occurred at 10º C within the five days of desiccation exposure. The SNK post-hoc analysis for mortality and exposure duration showed that exposure duration was significantly different with the highest mortality occurring at longer exposure durations. The data analysis for each of the SNK post-hoc analyses for each of the independent variables can be found in Appendix 5 (pages 55-56). Data on the shell length of mussels tested in the laboratory can be found in Appendix 2 (page 48). The average druse diameter in this experiment was 35.8 mm ± 4.4.

Table 2. Effects of Temperature, Relative Humidity and Exposure Duration on Mussel Mortality

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Temp	3	1435.722222	478.574074	966.09	<.0001
Humidity	2	9.375000	4.687500	9.46	0.0002
Day	2	375.125000	187.562500	378.63	<.0001
Temp*Humidity	6	28.236111	4.706019	9.50	<.0001
Temp*Day	6	596.486111	99.414352	200.69	<.0001
Humidity*Day	4	7.625000	1.906250	3.85	0.0058
Temp*Humidity*Day	12	22.930556	1.910880	3.86	<.0001

Multiple regression model

A multiple regression analysis was run with the variables of temperature, relative humidity and exposure duration through stepwise selection to determine the most predictive model between the independent variables to mortality. The first multiple regression model run used the entire dataset and found that temperature ($F=139.54$, $p < 0.0001$) and exposure duration ($F=53.08$, $p < 0.0001$) were significant and left in the model. Relative humidity was left out of the final model because it did not meet the significance level set for entry into the model. Stepwise regression shows that mortality rate (%) = -22.07 + 2.64* temp (º C) + 9.53 * day ($F_{2, 141} = 121.9$ $p < 0.0001$, $r^2 = 0.634$). Based on the regression model developed with the laboratory experiments and air temperature recorded in winter and summer (Tables 9 and 10), the predicted maximum duration to reach 100% mortality in summer and spring should be less than 0.1 and 6 days, respectively. In summer, 100% mortality was achieved by 12 hours. In spring, 100% mortality was achieved in 3 days. Therefore, the field measurements are in agreement with the regression model.

Table 3 shows the summary of the stepwise selection in the first multiple regression analysis.

Table 3. Summary of Stepwise Selection

Step	Variable Entered	Variable Removed	Label	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	Temp		Temp	1	0.4956	0.4956	54.6715	139.54	<.0001
2	Day		Day	2	0.1379	0.6336	3.4308	53.08	<.0001

Since relative humidity was not significant on quagga mussel mortality when exposed to higher temperatures (20, 30 and 40º C) a separate multiple regression analysis was conducted with data only collected for 10º C. The separate analysis found that both relative humidity ($F=12.70$, $p=0.0011$) and exposure duration ($F=42.99$, $p < 0.0001$) were significant and left in the model. Table 4 shows the summary of the stepwise selection for the multiple regression analysis for 10º C.

Table 4. Summary of Stepwise Selection, 10º C

Step	Variable Entered	Variable Removed	Label	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	Day		Day	1	0.5584	0.5584	13.6959	42.99	<.0001
2	Humid			2	0.1227	0.6811	3.0000	12.70	0.0011

At 10º C, 100% mortality was not achieved at any relative humidity during any of the exposure durations. At 20º C, 100% mortality was achieved at each relative humidity on day three. Druses of *D. bugensis* experienced 100% mortality at each value of relative humidity for both the temperatures of 30 and 40º C after one day. Table 5 shows the results of survivorship for each temperature, relative humidity and duration treatment.

Table 5. Percent survivorship of *D. bugensis*

10º C (50º F)			
% RH	Day 1	Day 3	Day5
20	97.5 (2.5)	62.5 (7.7)	32.5 (7.4)
50	97.5 (2.5)	97.5 (2.5)	35 (7.5)
80	100 (0)	97.5 (2.5)	70 (7.2)

30º C (86º F)			
% RH	Day 1	Day 3	Day5
20	0 (0)	0 (0)	0 (0)
50	0 (0)	0 (0)	0 (0)
80	0 (0)	0 (0)	0 (0)

20º C (68º F)			
% RH	Day 1	Day 3	Day5
20	100 (0)	0 (0)	0 (0)
50	97.5 (2.5)	0 (0)	0 (0)
80	100 (0)	0 (0)	0 (0)

40º C (104º F)			
% RH	Day 1	Day 3	Day5
20	0 (0)	0 (0)	0 (0)
50	0 (0)	0 (0)	0 (0)
80	0 (0)	0 (0)	0 (0)

Note: Standard error in paranthesis

Percent weight loss of *D. bugensis* exposed to desiccation

3-way ANOVA and SNK post-hoc test

The variables of temperatures, relative humidity and exposure duration explained 82% of the observed variance in percent weight loss among druses of *D. bugensis* (ANOVA $F_{34, 105} = 13.86$, $p < 0.0001$). The three-way ANOVA found that the two-way interaction between temperature and humidity ($F = 3.33$, $p = 0.0048$) and the two-way interaction between temperature and exposure duration ($F=4.21$, $p=0.0008$) were significant for percent weight loss of *D. bugensis*. Table 6 shows the results of the three-way ANOVA for percent weight loss. The SNK post-hoc analysis for percent weight loss and humidity showed no significant difference in percent water loss at 20% and 50% relative humidity with the lowest percent water loss occurring at 80% relative humidity. During the measurement process water spilled on four druses prior to post-treatment measurement and were left out of the analysis for 20% relative humidity (N=44). The SNK post-hoc analysis for percent weight loss and temperature showed that percent weight loss increased with temperature. The SNK post-hoc analysis for percent weight

loss and exposure duration showed that percent weight loss was greater at longer exposure durations. Appendix 8 (pages 61-64) shows that data analysis for each of the SNK post-hoc analyses for each of the independent variables.

Table 6. Effects of Temperature, Relative Humidity and Exposure Duration on Mussel Percent Weight Loss

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Temp	3	27186.08045	9062.02682	90.40	<.0001
Humidity	2	2180.54586	1090.27293	10.88	<.0001
Day	2	13900.08172	6950.04086	69.33	<.0001
Temp*Humidity	6	2003.79830	333.96638	3.33	0.0048
Temp*Day	6	2533.70497	422.28416	4.21	0.0008
Humidity*Day	4	178.57501	44.64375	0.45	0.7756
Temp*Humidity*Day	11	0.00000	0.00000	0.00	1.0000

Multiple regression model

A multiple regression analysis was run with the variables of temperature, relative humidity and exposure duration through stepwise selection to determine the most predictive model between the independent variables with percent weight loss. The multiple regression model found that temperature ($F=116.88$, $p < 0.0001$), relative humidity ($F=107.09$, $p < 0.0001$) and exposure duration ($F=12.12$, $p=0.0007$) were significant and left in the model. Table 7 shows the summary of the stepwise selection in the first multiple regression analysis.

Table 7. Summary of Stepwise Selection									
Step	**Variable Entered**	**Variable Removed**	**Label**	**Number Vars In**	**Partial R-Square**	**Model R-Square**	**C(p)**	**F Value**	**Pr > F**
1	Temp		Temp	1	0.4586	0.4586	127.904	116.88	<.0001
2	Day		Day	2	0.2375	0.6961	14.1195	107.09	<.0001
3	Humid			3	0.0249	0.7210	4.0000	12.12	0.0007

The relationship between both temperature and exposure duration with percent weight loss was directly proportional. Table 8 expresses the relationships between temperature, relative humidity and exposure duration.

Table 8. Percent weight loss of *D. bugensis*

10º C (50º F)			
% RH	Day 1	Day 3	Day5
20	Missing	44.9 (7.9)	64.6 (7.6)
50	21.9 (6.5)	37.2 (7.6)	61.1 (7.7)
80	16.4 (5.9)	30.7 (7.3)	41.2 (7.8)

30º C (86º F)			
% RH	Day 1	Day 3	Day5
20	43.7 (7.8)	66.0 (7.5)	65.1 (7.5)
50	46.2 (7.9)	66.6 (7.5)	65.4 (7.5)
80	48.1 (7.9)	64.1 (7.6)	66.4 (7.5)

20º C (68º F)			
% RH	Day 1	Day 3	Day5
20	14.0 (5.5)	26.9 (7.0)	35.6 (7.6)
50	11.4 (5.0)	19.5 (6.3)	37.6 (7.7)
80	13.0 (5.3)	18.7 (6.2)	21.6 (6.5)

40º C (104º F)			
% RH	Day 1	Day 3	Day5
20	31.5 (7.3)	62.8 (7.6)	62.9 (7.6)
50	36.7 (7.6)	62.6 (7.7)	62.0 (7.7)
80	32.9 (7.4)	48.6 (7.9)	49.4 (7.9)

Note: Standard error in paranthesis

Summer and Spring boat field validation

For the summer boat field validation, the surface temperature of the water at the time when the encrusted boat was pulled from out of the water was 27º C (81º F), the average air temperature and relative humidity the adult quagga mussels were exposed to were 34º C (94º F) and 26%, respectively. The results from the summer boat field validation experiment found that 100% mortality was reached within 6 hours (Table 9).

Table 9. Summer Boat Validation

Time	Water	Min ºC (ºF)	Max ºC (ºF)	Min R. humidity %	Max R. Humidity %	% Survivorship
0 hr	27.2 (81)	27.8 (82)	42.7 (109)	5.98	42.46	100
12 hrs	-	27.8 (82)	42.7 (109)	5.98	42.46	0
24 hrs	-	27.8 (82)	41.7 (107)	5.98	37.96	0
48 hrs	-	27.7 (80)	41.7 (107)	5.98	33.3	0
72 hrs	-	31.1 (88)	39.4 (103)	9.39	32.52	0
120 hrs	-	31.7 (89)	38.9 (102)	18.85	36.05	0
168 hrs	-	27.7 (102)	38.9 (102)	12.62	68.53	0

Note:Info from USGS Sentinel Island Surveillance

For the spring boat field validation, the surface temperature of the water at the time when the encrusted boat was pulled from the water was 12º C (53.6º F). The results of the spring boat field validation experiment found that 100% mortality was achieved after three days (Table 10). The average air temperature and relative humidity adult quagga mussels experienced was 20º C (68º F) and 26%, respectively.

Table 10. Spring Boat Validation

Time	Water	Min ºC (ºF)	Max ºC (ºF)	Min R. humidity %	Max R. Humidity %	% Survivorship
0 hr	12 (53.6)	12.2 (54)	27.8 (82)	12	35	100
30 min	-	12.2 (54)	27.8 (82)	12	35	100
1 hr	-	12.2 (54)	27.8 (82)	12	35	100
2 hrs	-	12.2 (54)	27.8 (82)	12	35	100
4 hrs	-	12.2 (54)	27.8 (82)	12	35	100
6 hrs	-	12.2 (54)	27.8 (82)	12	35	100
12 hrs	-	12.2 (54)	27.8 (82)	12	35	100
24 hrs	-	12.2 (54)	28.9 (84)	11	30	100
48 hrs	-	12.8 (55)	27.8 (82)	16	33	5
72 hrs	-	12.2 (54)	22.8 (73)	26	82	0

Note: Info from NOAA National Weather Service Forecast Office

ANCOVA model analysis shows that 1) both season and exposure duration were significant and affected mortality rate and 2) it took less time in summer than spring to reach 100% mortality ($F_{2, 12} = 18.48$, $p = 0.0002$). Data on the shell lengths of mussels collected from both boats can be found in Appendix 3 (page 49).

CHAPTER 5

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Discussion

Previous studies on desiccation resistance through aerial exposure or emersion have been primarily concerned with *D. polymorpha* (Zebra mussels) with limited data on *D. bugensis* (Quagga mussels) in the United States. Those previous studies have also involved dreissenid mussels east of the 100th meridian (Ricciardi et al., 1995; McMahon and Ussery, 1995; McMahon et al., 1993). Since the introduction and establishment of the dreissenid mussels in the Laurentian Great Lakes they have found their way across the entire United States as far as California. The United States is host to multiple biomes from east to west, which presents dreissenid mussels with different habitats to acclimate. The results from this study will provide more data that can be used to help in the efforts toward preventing further spread of *D. bugensis* into non-infested bodies of water in the Southwest. The results will also provide more information on temperature resistance of *D. bugensis*.

The spread of dreissenid mussels has been facilitated by multiple factors; the proximity between bodies of water, the dreissenid mussel's biology and physiology and its ability to survive outside of water, the discharge of ballast water, and the ability to travel down-stream. One thing that can be noted is that the major vector in aiding their spread is boats (McMahon, 2011). Currently, the bigger concern in the Southwest is *D. bugensis* with only one known body of water inhabited with *D. polymorpha*, the San Justo Reservoir in Califonia. As suggested by Ricciardi et al. (1995) with *D. polymorpha* the results of this study suggest that it is possible and probable for overland dispersal of

D. bugensis from neighboring bodies of water in the Southwest, if presented favorable conditions, for a short period of time (days). *D. bugensis* has been suggested to be less tolerant of aerial exposure compared with *D. polymorpha* (Ricciardi et al., 1995). *D. bugensis* survive longer outside of water in cooler, moister conditions which can be determined by the season depending on the state. Between the months of December and January, the maximum daily mean temperature in Las Vegas, NV, averages between 10 and 15.6º C (50 and 60º F, respectively). During the same months the maximum daily mean temperature in San Diego, CA, averages between 15.6 and 21.1º C (60 and 70º F, respectively). During the months of July through September the maximum daily mean temperature in Las Vegas, NV, averages between 37.8 and 43.3º C (100 and 110º F). In those same months the maximum daily mean temperature in San Diego, CA, averages between 21.1 and 26.6º C (70 and 80º F) (U.S. Dept. of Commerce, n.d.). The estimated time to travel from the Lake Mead Marina, Boulder City, NV to Lake Miramar, San Diego, CA is roughly less than 6 hours (Google Maps, 2012). Although Lake Miramar has been previously infested with *D. bugensis* it serves as an example of how overland dispersal is probable between two separate states. Due to the number of bodies of waters in California and the close proximity of those bodies of water the opportunity to inadvertently disperse *D. bugensis* to previously uncontaminated bodies of water to each other is highly probable. Anaheim Lake, Anaheim, CA is roughly 9 hours from either South Lake Tahoe (CA) or Clear Lake (CA) (Google Maps, 2012).

When temperature, relative humidity, and exposure duration were run in a multiple regression model the statistical results suggested that only temperature and exposure duration were significant factors on mortality. The two field experiments

(summer and spring) have confirmed that the model is robust. Although previous studies on aerial exposure and emersion have used different methodologies (temperature values, relative humidity values, and exposure times) the results have suggested that relative humidity had an effect on mortality along with temperature and exposure duration (McMahon et al., 1993; Ussery and McMahon, 1995; Ricciardi et al., 1995). In this study, *D. bugensis* did not survive longer than a day in 30 and 40º C, regardless of relative humidity. *D. bugensis* exposed to 20º C survived no longer than one day. Since the results for temperatures 20º C and higher nearly resulted in 100% mortality regardless of relative humidity and exposure a separate analysis was run for only 10º C alone. The separate analysis for 10º C found that relative humidity did have significance on morality in the overall model. These results agree with the results from previous studies found in the reviewed literature (McMahon et al., 1995; Ricciardi et al., 1995; McMahon et al., 1993). In this study, *D. bugensis* were tested in druses rather than individual mussels, which may have allowed a build up of anaerobic metabolites (ammonium accumulation). The higher the temperature that adult *D. bugensis* are exposed to results in less time required to achieve 100% mortality. *D. bugensis* have a greater survivorship at higher levels of relative humidity at temperatures below their upper incipient thermal temperature (< 30º C). *D. bugensis* submersed in water temperature of 30º C were found to not survive long periods of time (Spidle et al., 1995). Relative humidity may not be as important to *D. bugensis* once aerially exposed to temperatures of 30º C or above. As an observation, separate from this study, 4 druses of 10 mussels were placed on a mesh net above plastic supporters into a VWR 1000 mL PYREX beaker about 2-3 centimeters above distilled water. This chamber was left at room temperature (21.7 - 0.61º C) and left

open to allow the mussels fresh air supply. The observation found that 23% (7 out of 30) of *D. bugensis* had survived after three days at 95% relative humidity. (A druse of 10 of the 40 mussels found its way into the distilled water below the mesh net.) This observation agrees with the results found in Ricciardi et al. (1995) for 20º C, 95% relative humidity.

Percent weight loss of *D. bugensis* was measured in druses. The difference in the percent weight loss observed in druses showed lower weight loss occurred in higher relative humidity compared to greater weight loss in lower relative humidity. Percent weight loss was more pronounced at 10 and 20º C. The relationship stayed approximately proportional through the exposure duration with the lowest amount of weight loss occurring at 80% relative humidity and the highest amount of weight loss occurring at 20% relative humidity. The percent weight loss for *D. bugensis* at 30 and 40º C were similar where mussels experienced more than 30% and 40% of their weight by day one, respectively. By day three most of the druses had experienced more than 60% weight loss. Ricciardi et al. (1995) found that groups of mussels that experienced similar weight loss did not always result in similar mortality. In this experiment, 100% mortality occurred in most cases when there was 30% weight loss in druses. As suggested by Ricciardi et al. (1995), evaporative water loss due to desiccation may not be the only cause towards mortality. Druses exposed to 20º C survived almost entirely after one day at each relative humidity but ended in 100% mortality by day three. Percent weight loss experienced on day three of 20º C was 30% or more. *D. bugensis* can tolerate water temperatures of 20º C and in studies have been acclimated at that temperature (Ricciardi et al., 1995; Spidle et al., 1995; Beyer et al., 2011). Mortality at 20º C may have been

caused by multiple factors such as starvation or toxic build up of anabolic metabolites (ammonium accumulation). Similar to previous studies *D. bugensis* were observed to exhibit gaping behavior; the two valves spread apart and exposing their siphons (Ricciardi et al., 1995; McMahon and Payne, 1992). As mentioned by Ricciardi et al. (1995), ammonia may accumulate to toxic levels during prolonged aerial exposure or emersion.

The 100th meridian boat quarantine estimator was created to provide the minimum time a boat should remain out of water before being launched into an un-infested body of water. The data provided from the 100th meridian boat quarantine is based on the data from McMahon et al. (1993), which was data derived from testing zebra mussels. In the month of August it is suggested that a boat be kept from water between one to two days in southern Nevada. The summer boat field validation from this study agrees with the 100th meridian boat quarantine estimator. In August of 2011, *D. bugensis* were found to reach 100% mortality after 12 hours [35º C (95º F) and 24% relative humidity]. In the month of March it is suggested that a boat be kept from water between five to eight days in southern Nevada. The spring boat field validation from this study suggests that *D. bugensis* reach 100% mortality after three days [20º C (68º F) and 24% relative humidity].

Boat inspections are one of the most common and effective preventive measures taken by government agencies. Boat inspections are based on the status of the body of water; infested vs. non-infested. The extent of the inspection is set by guidelines and protocol's created by government agencies. Boater's should properly clean and dry their boats when leaving or before entering a body of water. The stakes are high considering it

only takes one mussel to infest a body of water. Over the past two decades there have been many studies conducted testing different approaches to control and maintain dreissenid populations and prevent the further spread of dreissenid mussels. Those approaches include physical and mechanical cleaning, chemical control, thermal control, and pressure wash. In order to help boaters avoid boat quarantines and avoid being turned away from entering a body of water there should be more alternative solutions to speed up the process of inspection. Aerial exposure is 100% effective given the right amount of time depending on the conditions. This study along with previous studies has underestimated the amount of time required to achieve 100% mortality. They do not take into consideration air flow and direct sunlight. A combination of approaches could be implemented in a watercraft decontamination station that allows a boat to pass through stages of cleaning similar to that of a drive thru car wash. In example, the initial stage allows chemical treatment of the boat. The second phase allows hot water ($\leq 60^{\circ}$ C) to wash off any chemicals left on the watercraft. The final phase allows the boat to be treated with high powered hot air to dry the watercraft. Although this is just an initial thought the possibility of implementing such watercraft decontamination station can be explored and there is a magnitude of data in the literature to consider.

Conclusion and Recommendations

Desiccation resistance has served as a preventive measure from inadvertent establishment of mussel communities and as a means of non-chemical control for dreissenid mussels. The 100th meridian boat quarantine estimator suggests quarantine times based on *D. polymorpha*, which has been shown in studies to be more resistant than

D. bugensis. Since the results of this study agree with the limited data found in the reviewed literature on *D. bugensis* and when compared with data on *D. polymorpha* in the literature it can be suggested that boat quarantine times should be based on the desiccation resistance of *D. polymorpha*.

Currently, *D. bugensis* imposes a greater threat than *D. polymorpha* in the southwestern United States. Although there is currently only one body of water with *D. polymorpha* present, protocols in boat quarantine should be developed on the more resistant of the two species as it already has with the 100th meridian boat quarantine estimator. The best control for these aquatic invasive species is the prevention of their onward spread into un-infested bodies of water. Aerial exposure or emersion as a preventive measure has the advantages of being cost-effective and providing a safer approach to environmental health by eliminating unnecessary use of chemical treatments. Aerial exposure or emersion can be 100% effective based on weather conditions and given the necessary amount of time. This method could be utilized in combination with other treatments depending on the season to allow boaters to spend less time decontaminating their boats or equipment. The stakes are high when considering new protocols and guidelines to implement at bodies of water used for leisure and recreational activities. It only takes one mussel to turn a non-infested body of water into an infested body of water.

This study allowed druses of adult *D. bugensis* to be exposed aerially to experimental conditions. Not taken into consideration are mussels that are attached to a boat are not exposed to still air. As a trailered boat is being hitch-towed the flow of air may have the effect of a hair dryer. The opportunity for mussels to be exposed to direct

sunlight also provides an effect that may cause mortality to occur at a quicker rate than mussels allowed desiccating in a chamber with no air current. The laboratory results of this study provide an approximate value rather than an absolute value. Other factors such as air flow and direct sunlight may cause mortality to occur quicker which in turn allows the results of this study to underestimate the amount of time to reach 100% mortality. More studies need to be conducted on dreissenid mussels found in bodies of water in the southwestern United States in order to assist government agencies and organizations in combating the infestation by dreissenid mussels. Currently there are no known infested bodies of water in the northwestern United States and this could be the next major are of concern.

APPENDIX 1

TEMPERATURE, RELATIVE HUMIDITY FLUCTUATION

	Std. Dev.	
10º C	0.64	
20%	11.18	
50%	11.52	
80%	13.23	
20º C	2.43	Average Temperature Flucation
20%	6.95	0.9575
50%	7.98	
80%	10.29	
30º C	0.72	
20%	7.17	Average Relative Humidity Fluctuation
50%	12.27	9.0075
80%	11.24	
40º C	0.04	
20%	2.46	
50%	4.81	
80%	8.99	

APPENDIX 2

EXPERIMENTAL SHELL LENGTH

Temp	Humidity	Day	mean	std	n
10	20	1	16.3	2.7	40
10	20	3	16.1	2.2	40
10	20	5	16.0	2.2	40
10	50	1	16.2	2.5	40
10	50	3	16.9	1.8	40
10	50	5	16.0	2.3	40
10	80	1	16.1	1.8	40
10	80	3	16.3	2.0	40
10	80	5	16.7	1.7	40
10	Control	1	16.6	2.4	40
10	Control	3	16.5	2.1	40
10	Control	5	15.3	1.6	40
20	20	1	16.6	2.1	40
20	20	3	16.8	2.3	40
20	20	5	16.8	2.2	40
20	50	1	17.3	2.4	40
20	50	3	17.4	2.8	40
20	50	5	16.6	1.9	40
20	80	1	16.5	2.5	40
20	80	3	17.2	2.5	39
20	80	5	16.7	1.9	40
20	Control	1	15.9	2.1	40
20	Control	3	16.0	2.0	40
20	Control	5	17.4	2.1	40
30	20	1	17.3	2.8	40
30	20	3	17.1	2.3	40
30	20	5	16.1	2.0	40
30	50	1	16.0	2.5	40
30	50	3	15.9	1.9	40
30	50	5	16.3	2.3	40
30	80	1	17.5	3.0	38
30	80	3	17.0	3.1	40
30	80	5	16.6	2.3	40
30	Control	1	16.4	2.4	40
30	Control	3	16.6	2.0	40
30	Control	5	16.4	2.0	40
40	20	1	16.1	3.0	40
40	20	3	17.8	2.9	40
40	20	5	16.5	2.1	40
40	50	1	17.7	2.3	40
40	50	3	17.4	3.0	40
40	50	5	16.8	2.4	40
40	80	1	17.7	3.3	40
40	80	3	15.5	1.9	40
40	80	5	16.9	2.1	40
40	Control	1	15.9	1.6	40
40	Control	3	16.3	1.7	40
40	Control	5	16.2	1.5	40

APPENDIX 3

FIELD VALIDATION SHELL LENGTH

Season	Hour	mean	std	n	
Summer	0	17.6	2.5	80	
Summer	12	17.6	2.3	80	
Summer	24	17.2	2.4	80	
Summer	48	17.0	2.2	80	
Summer	72	17.2	2.3	80	
Winter	0	17.3	2.5	80	
Winter	0.5	17.4	2.2	80	
Winter	1	16.5	2.4	80	
Winter	2	17.6	2.6	80	
Winter	4	17.1	2.7	80	
Winter	6	17.8	2.7	80	
Winter	12	18.1	2.2	80	
Winter	24	18.4	2.3	80	
Winter	48	17.9	2.1	80	
Winter	72	17.9	2.4	80	

APPENDIX 4

PERCENT WEIGHT LOSS

10 C (20%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	6.255	5.294	15.3637	14.0132	7.31077
Druse B	5.618	5.309	5.50018		
Druse C	5.24	4.606	12.0992		
Druse D	6.609	5.083	23.0897		
	Day 3		% Loss	MPWL	Stand. Dev.
Druse A	6.238	4.3	31.0676	26.9112	9.0479
Druse B	5.093	4.408	13.4498		
Druse C	5.596	3.907	30.1823		
Druse D	4.38	2.937	32.9452		
	Day 5		% Loss	MPWL	Stand. Dev.
Druse A	5.458	3.301	39.52	35.5575	14.4622
Druse B	5.055	2.812	44.3719		
Druse C	5.535	4.753	14.1283		
Druse D	7.34	4.095	44.2098		
10 C (50%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	7.714	6.925	10.2282	11.4262	1.76434
Druse B	7.243	6.266	13.4889		
Druse C	5.075	4.582	9.71429		
Druse D	4.693	4.117	12.2736		
	Day 3		% Loss	MPWL	Stand. Dev.
Druse A	5.328	4.532	14.9399	19.5265	7.95121
Druse B	6.78	5.016	26.0177		
Druse C	6.736	4.953	26.4697		
Druse D	5.909	5.278	10.6786		
	Day 5		% Loss	MPWL	Stand. Dev.
Druse A	5.899	3.669	37.803	37.6004	15.0123
Druse B	5.158	3.436	33.385		
Druse C	5.826	4.569	21.5757		
Druse D	5.656	2.396	57.6379		
10 C (80%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	5.769	5.241	9.15237	13.0014	3.36564
Druse B	5.307	4.513	14.9614		
Druse C	5.968	5.291	11.3438		
Druse D	6.883	5.744	16.548		
	Day 3		% Loss	MPWL	Stand. Dev.
Druse A	6.876	6.016	12.5073	18.6932	7.24103
Druse B	6.126	5.002	18.348		
Druse C	5.369	3.815	28.9439		
Druse D	5.503	4.679	14.9737		
	Day 5		% Loss	MPWL	Stand. Dev.
Druse A	6.876	4.107	40.2705	21.5838	16.3586
Druse B	6.126	4.797	21.6944		
Druse C	5.369	4.083	23.9523		
Druse D	5.503	5.48	0.41795		

20 C (20%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	6.733	Missing	Water spilled on druses =(		
Druse B	5.946	Missing	Water spilled on druses =(		
Druse C	6.821	Missing	Water spilled on druses =(		
Druse D	4.887	Missing	Water spilled on druses =(		
	Day 3		% Loss	MPWL	Stand. Dev.
Druse A	4.594	3.221	29.8868	44.8965	11.4921
Druse B	8.404	4.383	47.8463		
Druse C	6.887	2.919	57.6158		
Druse D	6.108	3.406	44.2371		
	Day 5		% Loss	MPWL	Stand. Dev.
Druse A	7.531	2.709	64.0287	64.6343	0.56034
Druse B	7.755	2.688	65.3385		
Druse C	6.375	2.27	64.3922		
Druse D	5.383	1.896	64.778		
20 C (50%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	9.154	7.524	17.8064	21.8982	12.4847
Druse B	6.893	6.19	10.1988		
Druse C	6.118	3.7	39.5227		
Druse D	4.939	3.948	20.0648		
	Day 3		% Loss	MPWL	Stand. Dev.
Druse A	4.555	4.349	4.5225	37.1741	30.9183
Druse B	5.114	4.213	17.6183		
Druse C	8.581	3.607	57.9653		
Druse D	7.953	2.498	68.5905		
	Day 5		% Loss	MPWL	Stand. Dev.
Druse A	5.741	2.063	64.0655	61.0726	5.08957
Druse B	5.182	1.974	61.9066		
Druse C	4.591	2.128	53.6484		
Druse D	5.709	2.017	64.6698		
20 C (80%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	5.646	4.44	21.3603	16.3758	7.03956
Druse B	4.465	4.119	7.74916		
Druse C	7.832	6.769	13.5725		
Druse D	6.529	5.039	22.8213		
	Day 3		% Loss	MPWL	
Druse A	9.965	6.586	33.9087	30.7442	12.7972
Druse B	5.489	3.621	34.0317		
Druse C	6.867	6.007	12.5237		
Druse D	5.142	2.956	42.5126		
	Day 5		% Loss	MPWL	
Druse A	7.166	3.955	44.8088	41.2418	3.40138
Druse B	5.066	2.979	41.1962		
Druse C	6.899	4.369	36.672		
Druse D	6.327	3.657	42.2902		

30 C (20%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	8.662	5.379	37.9012	31.5069	15.3742
Druse B	8.371	5.444	34.966		
Druse C	5.953	3.333	44.0114		
Druse D	5.334	4.846	9.14886		
	Day 3		% Loss	MPWL	Stand. Dev.
Druse A	6.455	2.585	59.9535	62.7944	2.42742
Druse B	6.072	2.072	65.8762		
Druse C	5.668	2.127	62.4735		
Druse D	5.678	2.108	62.8743		
	Day 5		% Loss	MPWL	Stand. Dev.
Druse A	4.289	1.503	64.9569	62.8783	6.48041
Druse B	4.823	1.809	62.4922		
Druse C	5.609	1.695	69.7807		
Druse D	4.681	2.14	54.2833		
30 C (50%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	7.903	4.96	37.239	36.6564	2.65248
Druse B	7.338	4.902	33.1971		
Druse C	4.009	2.543	36.5677		
Druse D	3.753	2.266	39.6216		
	Day 3		% Loss	MPWL	Stand. Dev.
Druse A	4.534	1.66	63.3877	62.6105	1.34745
Druse B	7.007	2.673	61.8524		
Druse C	4.035	1.568	61.14		
Druse D	5.451	1.959	64.0616		
	Day 5		% Loss	MPWL	Stand. Dev.
Druse A	4.413	1.95	55.8124	62.0384	4.55761
Druse B	6.994	2.325	66.7572		
Druse C	3.954	1.482	62.519		
Druse D	3.452	1.275	63.0649		
30 C (80%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	7.299	3.488	52.2126	32.8724	17.4384
Druse B	4.813	2.916	39.4141		
Druse C	4.929	3.505	28.8902		
Druse D	4.338	3.862	10.9728		
	Day 3		% Loss	MPWL	Stand. Dev.
Druse A	10.566	4.201	60.2404	48.616	11.193
Druse B	8.524	4.509	47.1023		
Druse C	4.185	2.767	33.8829		
Druse D	4.076	1.906	53.2385		
	Day 5		% Loss	MPWL	Stand. Dev.
Druse A	5.067	2.402	52.5952	49.4223	8.2777
Druse B	7.454	3.048	59.1092		
Druse C	6.186	3.717	39.9127		
Druse D	6.227	3.3581	46.0719		

40 C (20%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	4.907	3.259	33.5847	43.6614	10.5165
Druse B	5.544	3.109	43.9214		
Druse C	8.324	3.487	58.1091		
Druse D	5.652	3.446	39.0304		
	Day 3		% Loss	MPWL	Stand. Dev.
Druse A	9.829	3.308	66.3343	66.0443	3.62737
Druse B	8.41	2.522	70.0119		
Druse C	11.128	3.715	66.6157		
Druse D	6.09	2.362	61.2151		
	Day 5		% Loss	MPWL	Stand. Dev.
Druse A	6.331	2.204	65.1872	65.1102	3.57183
Druse B	5.401	1.636	69.7093		
Druse C	4.399	1.715	61.0139		
Druse D	7.006	2.485	64.5304		
40 C (50%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	6.004	3.511	41.5223	46.2397	5.60371
Druse B	7.397	3.927	46.9109		
Druse C	9.269	4.274	53.8893		
Druse D	7.571	4.343	42.6364		
	Day 3		% Loss	MPWL	Stand. Dev.
Druse A	5.496	1.893	65.5568	66.558	1.88354
Druse B	6.759	2.392	64.6101		
Druse C	9.964	3.099	68.898		
Druse D	10.127	3.325	67.167		
	Day 5		% Loss	MPWL	Stand. Dev.
Druse A	8.552	2.7	68.4284	65.4293	2.13669
Druse B	5.22	1.893	63.7356		
Druse C	6.138	2.205	64.0762		
Druse D	5.883	2.031	65.4768		
40 C (80%)	Before	After			
	Day 1		% Loss	MPWL	Stand. Dev.
Druse A	7.952	4.156	47.7364	48.111	6.8604
Druse B	11.026	6.488	41.1573		
Druse C	7.646	4.126	46.0371		
Druse D	6.289	2.672	57.5131		
			% Loss	MPWL	Stand. Dev.
Druse A	4.76	1.736	63.5294	64.1235	0.70914
Druse B	6.49	2.278	64.8998		
Druse C	5.372	1.96	63.5145		
Druse D	4.158	1.474	64.5503		
	Day 5		% Loss	MPWL	Stand. Dev.
Druse A	5.18	1.5	71.0425	66.3926	3.25108
Druse B	7.147	2.588	63.789		
Druse C	6.372	2.157	66.1488		
Druse D	6.196	2.194	64.5901		

APPENDIX 5

3-WAY ANOVA RESULTS: MORTALITY

The SAS System

The ANOVA Procedure

Class Level Information		
Class	**Levels**	**Values**
Temp	4	10 20 30 40
Humidity	3	20 50 80
Day	3	1 3 5

Number of Observations Read	144
Number of Observations Used	144

Dependent Variable: Mortality Mortality

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	35	2475.500000	70.728571	142.78	<.0001
Error	108	53.500000	0.495370		
Corrected Total	143	2529.000000			

R-Square	Coeff Var	Root MSE	Mortality Mean
0.978845	9.707938	0.703826	7.250000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Temp	3	1435.722222	478.574074	966.09	<.0001
Humidity	2	9.375000	4.687500	9.46	0.0002
Day	2	375.125000	187.562500	378.63	<.0001

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Temp*Humidity	6	28.236111	4.706019	9.50	<.0001
Temp*Day	6	596.486111	99.414352	200.69	<.0001
Humidity*Day	4	7.625000	1.906250	3.85	0.0058
Temp*Humidity*Day	12	22.930556	1.910880	3.86	<.0001

Student-Newman-Keuls Test for Mortality

Note: This test controls the Type I experimentwise error rate under the complete null hypothesis but not under partial null hypotheses.

Alpha	0.05
Error Degrees of Freedom	108
Error Mean Square	0.49537

Number of Means	**2**	**3**
Critical Range	0.2847745	0.3414205

Note: This test controls the Type I experimentwise error rate under the complete null hypothesis but not under partial null hypotheses.

Alpha	0.05
Error Degrees of Freedom	108
Error Mean Square	0.49537

Number of Means	**2**	**3**	**4**
Critical Range	0.3288292	0.3942384	0.4328959

Means with the same letter are not significantly different.			
SNK Grouping	**Mean**	**N**	**Temp**
A	10.0000	36	30
A			
A	10.0000	36	40
B	6.6944	36	20
C	2.3056	36	10

Note: This test controls the Type I experimentwise error rate under the complete null hypothesis but not under partial null hypotheses.

Alpha	0.05
Error Degrees of Freedom	108
Error Mean Square	0.49537

Number of Means	**2**	**3**
Critical Range	0.2847745	0.3414205

Means with the same letter are not significantly different.			
SNK Grouping	**Mean**	**N**	**Day**
A	8.8542	48	5
B	7.8542	48	3
C	5.0417	48	1

APPENDIX 6

MULTIPLE REGRESSION RESULTS: MORTALITY

The REG Procedure

Model: MODEL1

Dependent Variable: Mortality Mortality

Number of Observations Read	144
Number of Observations Used	144

Stepwise Selection: Step 1

Variable Temp Entered: R-Square = 0.4956 and C(p) = 54.6715

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	1253.47222	1253.47222	139.54	<.0001
Error	142	1275.52778	8.98259		
Corrected Total	143	2529.00000			

Variable	**Parameter Estimate**	**Standard Error**	**Type II SS**	**F Value**	**Pr > F**
Intercept	0.65278	0.61178	10.22685	1.14	0.2878
Temp	0.26389	0.02234	1253.47222	139.54	<.0001

Bounds on condition number: 1, 1

Stepwise Selection: Step 2

Variable Day Entered: R-square = 0.6336 and C(p) = 3.4308

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	2	1602.31597	801.15799	121.90	<.0001
Error	141	926.68403	6.57223		
Corrected Total	143	2529.00000			

Variable	Parameter Estimate	Standard Error	Type II SS	F Value	Pr > F
Intercept	-2.20660	0.65412	74.78894	11.38	0.0010
Temp	0.26389	0.01911	1253.47222	190.72	<.0001
Day	0.95313	0.13082	348.84375	53.08	<.0001

Bounds on condition number: 1, 4

All variables left in the model are significant at the 0.1500 level.

No other variable met the 0.1500 significance level for entry into the model.

Summary of Stepwise Selection									
Step	**Variable Entered**	**Variable Removed**	**Label**	**Number Vars In**	**Partial R-Square**	**Model R-Square**	**C(p)**	**F Value**	**Pr > F**
1	Temp		Temp	1	0.4956	0.4956	54.6715	139.54	<.0001
2	Day		Day	2	0.1379	0.6336	3.4308	53.08	<.0001

APPENDIX 7

MULTIPLE REGRESSION RESULTS: MORTALITY (10˚ C)

The REG Procedure

Model: MODEL1

Dependent Variable: Mortality Mortality

Number of Observations Read	36
Number of Observations Used	36

Stepwise Selection: Step 1

Variable Day Entered: R-square = 0.5584 and C(p) = 13.6959

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	170.66667	170.66667	42.99	<.0001
Error	34	134.97222	3.96977		
Corrected Total	35	305.63889			

Variable	**Parameter Estimate**	**Standard Error**	**Type II SS**	**F Value**	**Pr > F**
Intercept	-1.69444	0.69458	23.62540	5.95	0.0201
Day	1.33333	0.20335	170.66667	42.99	<.0001

Bounds on condition number 1, 1

Stepwise Selection: Step 2

Variable humid Entered: R-square = 0.6811 and C(p) = 3.0000

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	2	208.16667	104.08333	35.24	<.0001
Error	33	97.47222	2.95370		
Corrected Total	35	305.63889			

Variable	Parameter Estimate	Standard Error	Type II SS	F Value	Pr > F
Intercept	0.38889	0.83715	0.63740	0.22	0.6453
humid	-0.04167	0.01169	37.50000	12.70	0.0011
Day	1.33333	0.17541	170.66667	57.78	<.0001

Bounds on condition number: 1, 4

All variables left in the model are significant at the 0.1500 level.

All variables have been entered into the model

Summary of Stepwise Selection									
Step	**Variable Entered**	**Variable Removed**	**Label**	**Number Vars In**	**Partial R-Square**	**Model R-Square**	**C(p)**	**F Value**	**Pr > F**
1	Day		Day	1	0.5584	0.5584	13.6959	42.99	<.0001
2	humid			2	0.1227	0.6811	3.0000	12.70	0.0011

APPENDIX 8

3-WAY ANOVA: PERCENT WEIGHT LOSS

The SAS System

The ANOVA Procedure

Class Level Information		
Class	**Levels**	**Values**
Temp	4	10 20 30 40
Humidity	3	20 50 80
Day	3	1 3 5

Number of Observations Read	144
Number of Observations Used	140

Dependent Variable: W_Loss_Percent W_Loss_Percent

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	47236.05663	1389.29578	13.86	<.0001
Error	105	10525.38451	100.24176		
Corrected Total	139	57761.44114			

R-Square	Coeff Var	Root MSE	W_Loss_Percent Mean
0.817778	23.40183	10.01208	42.78332

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Temp	3	27186.08045	9062.02682	90.40	<.0001
Humidity	2	2180.54586	1090.27293	10.88	<.0001
Day	2	13900.08172	6950.04086	69.33	<.0001

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Temp*Humidity	6	2003.79830	333.96638	3.33	0.0048
Temp*Day	6	2533.70497	422.28416	4.21	0.0008
Humidity*Day	4	178.57501	44.64375	0.45	0.7756
Temp*Humidity*Day	11	0.00000	0.00000	0.00	1.0000

Student-Newman-Keuls Test for W_Loss_Percent

Note: This test controls the Type I experimentwise error rate under the complete null hypothesis but not under partial null hypotheses.

Alpha	0.05
Error Degrees of Freedom	105
Error Mean Square	100.2418
Harmonic Mean of Cell Sizes	46.58824

Note: Cell sizes are not equal.

Number of Means	2	3
Critical Range	4.1132344	4.9317885

Means with the same letter are not significantly different.			
SNK Grouping	**Mean**	**N**	**Humidity**
A	47.092	44	20
A			
A	44.019	48	50
B	37.598	48	80

Note: This test controls the Type I experimentwise error rate under the complete null hypothesis but not under partial null hypotheses.

Alpha	0.05
Error Degrees of Freedom	105
Error Mean Square	100.2418
Harmonic Mean of Cell Sizes	34.90909

Note: Cell sizes are not equal.

Number of Means	2	3	4
Critical Range	4.7517362	5.6973553	6.2563265

Means with the same letter are not significantly different.			
SNK Grouping	**Mean**	**N**	**Temp**
A	59.074	36	40
B	49.933	36	30
C	39.755	32	20
D	22.035	36	10

Note: This test controls the Type I experimentwise error rate under the complete null hypothesis but not under partial null hypotheses.

Alpha	0.05
Error Degrees of Freedom	105
Error Mean Square	100.2418
Harmonic Mean of Cell Sizes	46.58824

Note: Cell sizes are not equal.

Number of Means	2	3
Critical Range	4.1132344	4.9317885

Means with the same letter are not significantly different.			
SNK Grouping	**Mean**	**N**	**Day**
A	52.747	48	5
B	45.724	48	3
C	28.706	44	1

APPENDIX 9

MULTIPLE REGRESSION RESULTS: PERCENT WEIGHT LOSS

The REG Procedure

Model: MODEL1

Dependent Variable: W_Loss_Percent W_Loss_Percent

Number of Observations Read	144
Number of Observations Used	140
Number of Observations with Missing Values	4

Stepwise Selection: Step 1

Variable Temp Entered: R-square = 0.4586 and C(p) = 127.9044

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	26488	26488	116.88	<.0001
Error	138	31274	226.61970		
Corrected Total	139	57761			

Variable	**Parameter Estimate**	**Standard Error**	**Type II SS**	**F Value**	**Pr > F**
Intercept	12.19563	3.10216	3502.50337	15.46	0.0001
Temp	1.21656	0.11253	26488	116.88	<.0001

Bounds on condition number: 1, 1

Stepwise Selection: Step 2

Variable Day Entered: R-square = 0.6961 and C(p) = 14.1195

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	2	40209	20104	156.92	<.0001
Error	137	17553	128.12142		
Corrected Total	139	57761			

Variable	Parameter Estimate	Standard Error	Type II SS	F Value	Pr > F
Intercept	-6.83894	2.97050	679.11149	5.30	0.0228
Temp	1.23060	0.08462	27096	211.49	<.0001
Day	6.11073	0.59049	13721	107.09	<.0001

Bounds on condition number: 1.0003, 4.001

Stepwise Selection: Step 3

Variable Humid Entered: R-square = 0.7210 and C(p) = 4.0000

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	3	41645	13882	117.14	<.0001
Error	136	16116	118.50321		
Corrected Total	139	57761			

Variable	Parameter Estimate	Standard Error	Type II SS	F Value	Pr > F
Intercept	0.26295	3.51042	0.66490	0.01	0.9404
Temp	1.22585	0.08139	26880	226.83	<.0001
Humid	-0.13193	0.03790	1436.19743	12.12	0.0007
Day	6.02158	0.56847	13296	112.20	<.0001

Bounds on condition number: 1.0023, 9.0154

All variables left in the model are significant at the 0.1500 level.

All variables have been entered into the model.

Summary of Stepwise Selection									
Step	**Variable Entered**	**Variable Removed**	**Label**	**Number Vars In**	**Partial R-Square**	**Model R-Square**	**C(p)**	**F Value**	**Pr > F**
1	Temp		Temp	1	0.4586	0.4586	127.904	116.88	<.0001
2	Day		Day	2	0.2375	0.6961	14.1195	107.09	<.0001
3	Humid			3	0.0249	0.7210	4.0000	12.12	0.0007

BIBLIOGRAPHY

100th Meridian Initiative. (2011). Zebra/quagga mussel information. Available from http://www.100thmeridian.org.

Ackerman, J.D., Sim, B., Nichols, S.J. and Claudi, R. (1994). A review of the early life history of zebra mussels (*Dreissena polymorpha*): comparisons with marine bivalves. *Canadian Journal of Fisheries and Aquatic Sciences*. 72:1169-1179.

Armistead, D.C. (1995).Tolerances of zebra mussels to various temperatures in the Mississippi and Ohio Rivers, 1988-1992. Technical Report ZMR-1-32, U.S. Army Corps of Engineers Waterways Experiment Station. Vicksburg, MS.

Aquatic Nuisance Species (ANS) Task Force. (2012). Aquatic nuisance species information. Available from http://anstaskforce.gov/default.php

Benson, A.J. and Raikow, D. (2011). Dreissena polymorpha. USGS Nonindigenous Aquatic Species Database, Gainesville, FL. http://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=5 Revision Date: 7/8/2010

Benson, A.J. Richerson, M.M. and Maynard, E. (2011). Dreissena rostiformis bugensis. USGS Nonindigenous Aquatic Species Database, Gainesville, FL. http://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=95 Revision Date: 9/14/2011

Bially, A. and MacIsaac, H.J. (2000). Fouling mussels (Dreissena spp.) colonize soft sediments in Lake Erie and facilitate benthic invertebrates. *Freshwater Biology*. 43:85-97.

Beyer, J. Moy, P. and De Stasio, B. (2011). Acute upper thermal limites of invasive invertebrates: hot water treatment to prevent upstream transport of invasive species. Environmental Management 47:67-76.

Boelman, S.F., Neilson, F.M., Dardeau, E.A. and Cross T. (1996) "Zebra mussel (*dreissena polymoprha*) control handbook for facility operators, first edition," Miscellaneous Paper EL-91-1, U.S. Army Corps of Engineers Waterway Experiment Station, Vicksburg, MS.

Britton, D., Brown, E., Heimowitz, P., Morse, J., Pitman, B., Ryce, E., Smith, R., Williams, E. (2010). Quagga-zebra mussel action plan for western U.S. waters. The Western Regional Panel on Aquatic Nuisance Species. Retrieved from http://www.anstaskforce.gov/QZAP/rQZAP_FINAL_Feb2010.pdf on January 18, 2012.

Bruner, K.A., Fisher, S.W. and Landrum, P.F. (1994). The role of the zebra mussel, *Dreissena polymorpha*, in contaminant cycling: I. The effect of body size and lipid

content on the bioconcentration of PCBs and PAHs. Journal of Great Lakes Research 20: 725-734.

Caraco, N.F., Cole, J.J., Raymond, P.A., Strayer, D.L., Pace, M.L., Findlay, S. and Fischer, D.T. (1997). Zebra mussel invasion in a large, turbid river: phytoplankton response to increased grazing. Ecology 78: 588-602.

Carlton J.T. (1993). Dispersal mechanism of the zebra mussel (*Dreissena polymorpha*). In Napela, T.F. & Schloesser, D.W. (Eds.), Zebra Mussel's: Biology, Impacts and Control (677-697). Chelsea, MI: Lewis Publishers.

Claudi R. and Mackie G.L. (1994). Practical Manual for Zebra Mussel Monitoring and Control. Lewis Publishers, Boca Raton, FL.

Comeau, S., Rainville, S., Baldwin, W., Austin, E., Gerstenberger, S., Cross, C., Wong, W.H. (2011). Susceptibility of quagga mussels (Dreissena rostriformis bugensis) to hot-water sprays as a means of watercraft decontamination. Biofouling 27(3):267-274.

Cotruvo, J.A. & Regelski, M. (1989).Issues in developing national primary drinking water regulations for disinfection and disinfection by-products. In E.J. Calabrese, C.E.

Claxton, W.T., Martel A., Dermott R.M., Boulding, E.G. (1997). Discrimination of field-collected juveniles of two introduced dreissenids (Dreissena polymorpha and Dreissena bugenisis) using mitochondrial DNA and shell morphology. Canadian Journal of Zoology 54:1280-1288.

Crosier, D.M. & Molloy, D.P. (2001). Zebra Mussel Life History and Biology. Zebra Mussel Information System. Retrieved 16 Jan 2012. http://el.erdc.usace.army.mil/zebra/zmis/.

Cross, C.L., Wong, W.H.s, Che,T. (2011). Estimating carrying capacity of quagga mussels (*Dreissena rostriformis bugensis*) in a natural system: A case study of the Boulder Basin of Lake Mead, Nevada-Arizona. Aquatic Invasions. 6:141-147.

Domm, S., McCauley, R.W., and Kott, E. (1993). Physiological and taxonomic separation of two dreissenid mussels in the Laurentian Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. 50:2294-2298.

Elderkin, C.L. and Klerks, P.L. (2005). Variation in thermal tolerance among three Mississippi River populations of the zebra mussel, Dreissena polymorpha. Journal of Shellfish Research 24: 221–226.

Gillis, P.L., and Mackie, G.L. (1994). Impact of the zebra mussel, *Dreissena polymorpha*, on populations of Unionidae (Bivalvia) in Lake St. Clair. Can. J. Zool. **72**: 1260–1271.

Griffiths R.W., Schloesser D.W., Leach J.H. and Kovalak W.P. (1991) Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes Region. Canadian Journal of Fisheries and Aquatic Sciences. 48(8):1381-1388.

Gerstenberger S.L., Powell S., McCoy M. (2004). The 100th Meridian Initiative in Nevada: Assessing the Potential Movement of the Zebra Mussel to the Lake Mead National Recreation Area, Nevada, USA. Retrieved from http://www.100thmeridian.org/Documents/Zebra%20Mussels%20Nevada.pdf on January 18, 2011.

Google Maps. (2012). [Anahiem Lake, Anaheim, CA to South Lake Tahoe, CA.] [Street map]. Retreieved from https://maps.google.com/maps?saddr=Anaheim+Lake,+Anaheim,+CA&daddr=South+Lake+Tahoe,+CA&hl=en&sll=36.013491,-114.965401&sspn=0.075189,0.075016&geocode=Fby7BAIdR8j5-CnjKzWYu9bcgDGNDUSSxR-eNQ%3BFfkSUgIdJC_Z-CnhT6XuiYWZgDGOkSP7Ri8b3Q&oq=South+Lake+Tahoe&mra=ls&t=m&z=7

Google Maps. (2012). [Anaheim Lake, Anaheim, CA to Clear Lake, CA.] [Street map]. Retrieved from https://maps.google.com/maps?saddr=Anaheim+Lake,+Anaheim,+CA&daddr=Clear+Lake,+CA&hl=en&ll=38.869652,-122.872467&spn=1.15794,1.200256&sll=36.412935,-118.69125&sspn=9.569609,9.602051&geocode=Fby7BAIdR8j5-CnjKzWYu9bcgDGNDUSSxR-eNQ%3BFQ77UwIdWb-t-CnnhucBkO2DgDF1vGbJnXKOtg&oq=Clear+Lake,+CA&mra=ls&t=m&z=10

Google Maps. (2012). [Lake Mead Marina, Boulder City, NV to Lake Miramar, San Diego, CA.] [Street map]. Retrieved from https://maps.google.com/maps?saddr=Lake+Mead+Marina,+Boulder+City,+NV&daddr=Lake+Miramar,+San+Diego,+CA&hl=en&ll=35.971338,-115.081787&spn=0.60183,0.600128&sll=38.502032,-117.02306&sspn=18.591183,19.204102&geocode=FcWzJQId_bko-SFaRASp40ni8imxq2gw-CzJgDFaRASp40ni8g%3BFfg-9gEd_jQF-SmHCTNHPvnbgDEOmvenu4avjw&oq=Lake+Mira&t=m&mra=ls&z=11

Hebert, P.D.N., Muncaster, B.W. and Mackie, G.L. (1989). Ecological and genetic studies on *Dreissena polymorpha* (Pallas): A new mollusk in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. 46(9):1587-1591.

Holeck, K.T., Mills, E.L., MacIsaac, H.J., Dochoda, M.R., Colautti, R.I. and Ricciardi, A. (2004). Bridging troubled waters: biological invasions, transoceanic shipping, and the Laurentian Great Lakes. Bioscience 54: 919-929.

Iwanyzki, S., and McCauley, R.W. (1993). Upper lethal tem- peratures of adult zebra mussels (Dreissena polymorpha). In Zebra mussels: biology, impacts, and controls. Edited by T.F. Nalega and D.W. Schloesser. Lewis Publishers, Inc., Boca Raton, Fla. pp. 667-673.

Jenner, H.A., and Janssen-Mommen, J.P.M. (1993). Monitoring and control of Dreissena polymorpha and other macrofouling bivalves in the Netherlands. In Zebra mussels: biology, impacts, and control. Edited by T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Inc., Boca Raton, Fla. pg. 537-554.

Johnson, L.E. and Carlton, J.T. (1996) Post-establishment spread in large-scale invasions: dispersal mechanisms of the zebra mussel *Dreissena polymorpha*. Ecology 77: 1686–1690.

Johnson L.E., Ricciardi A., Carlton J.T. (2001). Overland dispersal of aquatic invasive species: a risk assessment of transient recreational boating. Ecological Applications 11:1789-1799.

Karatayev, A.Y., Burlakova, L.E. and Padilla, D.K. (1997). The effects of *Dreissena polymorpha* (Pallas) invasion on aquatic com- munities. Eastern European Journal of Shellfish Research 16: 187–203.

LaBounty, J.F. and Roefer, P. (2007). Quagga mussels invade Lake Mead. LakeLine 27:17-22.

Leung, B., Lodge, D.M. Finnoff, D., Shogren, J.F., Lewis, M. & Lamberti, G. (2002) An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. *Proceedings of the Royal Society, Series B*, **269**, 2407–2413.

Lodge, D.M. (1993). Biological invasions: lessons for ecology. Trends in Ecology and Evolution. 8(4):133-137.

MacIsaac, H.J. (1996). Potential and Abiotic and Biotic Impacts of Zebra Mussels on the Inland Waters of North America. *American Zoology*. 36:287-299.

Mackie, G.L. (2004). *Applied Aquatic Ecosystem Concepts*. Kendall/Hunt Publishing Company, Dubuque, IO.

Mackie, G.L. and Schloesser, D.W. (1996). Comparative biology of zebra mussels in Europe and North America: An overview. *American Zoologist*. 36:244-258.

Mackie, G.L. and Claudi, R. (2009). Monitoring and Control of Macrofouling Mollusks in Fresh Water Systems, Second Edition. CRC Press, Boca Raton, FL.

Madge, D.S. (1961). The control of relative humidity with aqueous solutions of sodium hydroxide. Entomol. Exp. Appl. 4: 143-147.

May, B. and Marsden, J.E. (1992). Genetic identification and implications of another invasive species of Dreissenid mussel in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. 49:1501-1506.

McMahon, R.F. (1991). Mollusca: bivalvia. In: *Ecology and Classification of North American Freshwater Invertebrates* (Eds Thorpe J.H. and Covich A.P.), pp. 315-390. Academic Press, New York.

McMahon, R.F. and Payne B.S. (1992). Effects of temperature and relative humidity on desiccation resistance in zebra mussels (Dreissena polymorpha): Is aerial exposure a viable control option. J. Shellfish Res. 11:233.

McMahon, R.F., Ussery, T.A., and Clarke, M. (1993). Use of emersion as a zebra mussel control method. Technical Report EL-93-1, U.S. Army Corps of Engineers Waterway Experiment Station. Vicksburg, MS.

McMahon, R.F. and Ussery, T.A. (1995). Thermal tolerance of zebra mussels (Dreissena polymorpha) relative to rate of temperature increase and acclimation temperature. Vicksburg (MS): US Army Corps of Enginners, Waterways Experiment Station. p. 1-21.

McMahon, R.F. (1996). The physiological ecology of the zebra mussel, *Dreissena polymorpha*, in North America and Europe. *American Zoologist*. 36:244-258.

McMahon, R.F. (2011). Quagga mussel (*Dreissena rostriformis bugensis*) population structure during the early invasion of Lakes Mead and Mohave January-March 2007. Aquatic Invasions 6: 131-140

Mills, E.L., Dermott, R.M., Roseman, E.F., Dustin, D., Mellina, E., Conn, D.B., & Spidle, A.P. (1993). Colonization, ecology, and population structure of the ␣quagga␣ mussel (Bivalva, Dressenidae) in the lower Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. 50:2305-2314.

Mills, E.L., Rosenberg, G., Spidle, AP, Ludyanskiy, M., Pligin Y., and May, B. (1996). A review of the biology and ecology of the quagga mussel (Dreissena bugensis), a second species of freshwater dreissenid introduced to North America. *American Zoology*. 25(1):187-197.

Molles, M. C. (2005). *Ecology: Concepts and applications*. (3rd ed., pp. 2-12). New York, NY: McGraw-Hill Higher Education.

Morse, J.T. (2009). Assessing the effects of application time and temperature on the efficacy of hot-water sprays to mitigate fouling by Dreissena polymorpha (zebra mussels Pallas). Biofouling 23:605-610.

Morton, B. (1993). The anatomy of *Dreissena polymorpha* and the evolution and success of the heteromyarian from the Dreissenoidea. In Nalepa T.F. and Schloesser D.W., eds., *Zebra mussels" Biology, impacts, and control*, pp. 185-215. Lewis Publishers Chelsea, MI.

Mueting, S.A., Gerstenberger, S.L. and Wong, W.H. (2010). An evaluation of artificial substrates for monitoring the quagga mussel (Dreissena bugensis) in Lake Mead, Nevada-Arizona. *Lake and Reservoir Management*. 26(4):283-292.

NDOW AIS Fact Sheet. (2011). Aquatic Invasive Species (AIS) Fact Sheet AB167 2011 Nevada Department of Wildlife. Retrieved from www.leg.state.nv.us/Session/76th2011/Exhibits/.../ANRAM339H.pdf on February 2, 2012.

O'Neill, C. 1993. Control of zebra mussels in residential water systems. New York Seat Grant Fact Sheet. http://systecoreinc.com/files/Zebra_Mussels_and_Copper.doc

Payne, B.S. (1992a). Freeze survival of aerially exposed zebra mussels. Technical Report ZMR-2-09, U.S. Army Corps of Engineers Waterway Experiment Station, Vicksburg, MS.

Payne, B.S. (1992b). Aerial exposure and mortality of zebra mussels. Technical Report ZMR-2-10, U.S. Army Corps of Engineers Waterway Experiment Station, Vicksburg, MS.

Pimentel, D., Zuniga, R. and Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273-288.

Poulin, R., Paterson, R.A., Townsend, C.R., Tompkins, D.M. and Kelly, D.W. (2011). Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. Freshwater Biology 50: 676-688.

Rajagopal ,S., van der Velde, G., van der Gaag, M. and Jenner, H.A. (2005). Factors influencing the upper temperature tolerances of three mussel species in a brackish water canal: size, season, and laboratory protocols. Biofouling 21:87–97.

Ram, J.L., Fong, P.P. and Garton, D.W. (1996). Physiological Aspects of Zebra Mussel Reproduction: Maturation, Spawning, and Fertilization. *American Zoology*. 36:326-338.

Ricciardi, A., Serrouya, R., and Whoriskey, F.G. (1995). Aerial exposure tolerance of zebra and quagga mussels (Bivalvia: Dreissenidae): implications for overland dispersal. *Canadian Journal of Fisheries and Aquatic Sciences*. 52:470-477.

Ricciardi, A., Whoriskey, F.G. and Rasmussen J.B. (1997). The role of the zebra mussel (*Dreissena polymorpha*) in structuring macroinvertebrate communities on hard substrata. Canadian Jounral of Fisheries and Aquatic Sciences 54: 2596-2608.

Ricciardi, A., Neves, R.J., Rasmussen, J.B. (1998). Impending Extinctions of North American Freshwater Mussels (Unionoida) Following the Zebra Mussel (*Dreissena polymorpha*) Invasion. *Journal of Animal Ec*ology; Vol. 67, pp. 613-619.

Schloesser, D.W., and Nalepa, T.F. (1994). Dramatic decline of unionid bivalves in offshore waters of western Lake Erie after infestation by the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. **51**: 2234–2242.

Spidle, A.P., Mills, E.L., May, B. (1995). Limits to tolerance of temperature and salinity in the quagga mussel (Dreissena bugensis) and the zebra mussel (Dreissena polymorpha). Canadian Journal of Fisheries and Aquatic Sciences 52:2108-2119.

Sprecher, S. and Getsinger, K.D. (2000). Zebra Mussel Chemical Control Guide. U.S. Army Corps of Engineers. Washington, DC. ERDC/EL TR-00-1.

Stop Aquatic Hitchhikers. (2011). Zebra/quagga mussel information. Available from http://www.protectyourwaters.net/

United States Bureau of Reclamation (USBR). (2010). Inspection and cleaning manual for equipment and vehicles to prevent the spread of invasive species. Retrieved from: http://www.usbr.gov/mussels/prevention/docs/EquipmentInspectionandCleaningManual2010.pdf on February 2, 2012.

U.S. Department of Commerce (n.d). National Oceanic and Atmospheric Administration. Earth System Research Laboratory. Physical Sciences Division. Retrieved from http://www.esrl.noaa.gov/psd/data/usclimate/states.fast.html on August 25, 2012.

Watters, A. (2011). Effectiveness of EarthTec ® on killing invasive quagga mussels (*dreissena rostiformis bugensis*) and preventing their colonization in the Western U.S. Thesis. University of Nevada, Las Vegas. 84 pp.

Wong, W.H. and Gerstenberger, S.L. (2011). Quagga mussels in the western United States: Monitoring and Management. Aquatic Invasions 6: 125-129.

Zhulidov, A.V., Pavlov, D.F., Nalepa, T.F., Scherbina, G.H., Zhulidov, D.A., Gurtovaya, T.Y. (2006). Relative distribution of Dreissena bugenisis and Dreissena polymorpha in the Lower Don River System, Russia. Internationale Revue der Gesamten Hydrobiologie 89:326-333.

Zook, B. and Phillips S. (2009). Recommended Uniform Minimum Protocols and Standards for Watercraft Interception Programs for Dreissenid Mussels in the Western United States. Western Regional Panel on Aquatic Nuisance Species [Internet]. [cited 2012 Feb 15]. Available from: http://www.aquaticnuisance.org/wordpress/wp-content/uploads/2009/01/Recommended-Protocols-and-Standards-for-Watercraft-Interception-Programs-for-Dreissenid-Mussels-in-the-Western-United-States-September 8.pdf

VITA

Graduate College
University of Nevada, Las Vegas

Matthew Kappel

Degrees:
Bachelor of Science, Biology, 2007
University of Nevada, Las Vegas

Thesis Title: *Dreissena rostiformis bugensis*: Aerial Exposure of Adult Quagga Mussels Found in Lake Mead as a Preventive Measure of Overland Dispersal in the Western United States

Thesis Examination Committee:
Chairperson, David Wong, Ph.D.
Co-Chair, Shawn Gerstenberger, Ph.D.
Committee Member, Paulo Pinheiro, Ph.D.
Graduate Faculty Representative, Steen Madsen, Ph.D.