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Understanding Basin Specific Life History Characteristics of Lake Mead Quagga Mussels (Dreissena bugensis) and a Potential Treatment Using UV Radiation in Laboratory Studies

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UNDERSTANDING BASIN SPECIFIC LIFE HISTORY CHARACTERISTICS OF LAKE MEAD QUAGGA MUSSELS \textit{(Dreissena bugensis)} AND A POTENTIAL TREATMENT USING UV RADIATION IN LABORATORY STUDIES

by

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Bachelor of Arts
Lewis & Clark College, 2004

A thesis submitted in partial fulfillment of the requirements for the

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ABSTRACT

Understanding Basin Specific Life History Characteristics of Lake Mead Quagga Mussels (*Dreissena bugensis*) and a Potential Treatment Using UV Radiation in Laboratory Studies

by

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The quagga mussel (*Dreissena bugensis*) is an aquatic invasive species that is spreading throughout Lake Mead and other western waterways. Unlike their native waters in Eurasia, Lake Mead exhibits year round warm temperatures, high calcium levels and a lack of natural predators, all of which are very favorable conditions for their growth and spread. *Dreissena bugensis* reproduce and colonize hard surfaces rapidly, where they filter large amounts of water. They disrupt the aquatic food chain and interfere with infrastructure that is exposed to lake water. There is an urgent need to understand *Dreissena bugensis* life history characteristics within this new habitat to help managers make decisions. Not only does Lake Mead present opportunities for *Dreissena bugensis* to cause ecological damage, but economic damage to infrastructure is also major concern. Lake Mead is made up of several unique basins, which present unique conditions for *Dreissena bugensis* and unique challenges to managers. This study sought to characterize Lake Mead basin specific characteristics and respective *Dreissena bugensis* growth rates in order to shed light on how *Dreissena bugensis* growth might vary from basin to basin.
Water quality data was analyzed to characterize two different basins with a focus on nutrients fundamental to the aquatic food chain, including nitrogen (N), phosphorus (P), total organic carbon (TOC) and chlorophyll a. Additionally, *Dreissena bugensis* growth rates from each of those basins were measured in laboratory experiments. Boulder Basin had significantly higher nutrient levels and Overton Arm had significantly higher chlorophyll a levels. Overton Arm yielded higher *Dreissena bugensis* somatic growth rates. This adds to our knowledge of how basin specific characteristics may be influencing the interaction between invasive *Dreissena bugensis* and their recently colonized habitats in a complex reservoir.

There is a major need for improved methods of treating invasive mussels at Lake Mead and other invaded waterways. The development of non-chemical treatments, such as ultraviolet (UV) radiation, is an important area in need of research because many chemical treatments, such as chlorine, produce harmful byproducts. The first step in developing UV as a potential treatment for *Dreissena bugensis* is to determine the amount and intensity needed to kill *Dreissena bugensis* at various life stages. The second part of this study sought to model, through dose-response functions, the quantity of UV needed to obtain high mortality rates of adult, juvenile and veliger *Dreissena bugensis*.

*Dreissena bugensis* were exposed to different intensities of UV radiation in laboratory experiments. Chronic (long-term) exposure was administered to adult and juvenile mussels, with mortality monitored daily. Veligers were administered acute (short-term) exposure and mortality was observed post-exposure. Dose-response functions were fitted to the resulting data to represent the relationship between the dose (time and intensity) and the resulting mortality. The Lethal Dose-50 (LD$_{50}$), or median lethal dose, is
the lethal dose needed for 50% mortality. The LD$_{50}$ was calculated from the dose-response functions; the LD$_{50}$ values can be used to compare *Dreissena bugensis* with other species. The quantification of UV needed for high mortality rates in *Dreissena bugensis* found in this study can be used in engineering applications for treating *Dreissena bugensis*. The LD$_{50}$ values were estimated as 44,000 mJ/cm$^2$, 11,000 mJ/cm$^2$ and 860 mJ/cm$^2$ for adult, juvenile and veliger *Dreissena bugensis*, respectively.
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CHAPTER 1—INTRODUCTION

Invasive species become established within new ecosystems and cause environmental damage. When species are introduced to ecosystems with organisms that they have no common evolutionary history, predator-prey and resource competition dynamics are altered (Freeman and Byers, 2006). Invasive species outcompete native species, alter habitats and act as unnatural predators to native species (Mack et al., 2000). Once invasive species have invaded a new environment it is extremely difficult to eradicate them; developing and improving management strategies is important to minimize economic damage to infrastructure and resources (Mack et al., 2000). The economic cost of invasive species is so high and widespread that it is considered “incalculable” and ecological disruptions are comparable to global warming (Mack et al., 2000). The ecological problem of invasive species becomes even more severe in the context of climate change. As native species cannot move fast enough into areas with suitable temperatures, conversely, invasive species spread quickly and can better adapt to conditions resulting from climate change than native species (Hulme, 2012).

*Dreissena bugensis* reproduce at high rates resulting in rapid population expansion (Wittmann et al., 2010). By filtering large volumes of water, they remove seston and algae, making it unavailable in the food chain to native aquatic species, thus disrupting aquatic ecosystems (Wittmann, et al. 2010). Economic damage caused by *Dreissena bugensis* extends to sectors including water treatment, power generation, industry and agriculture (Sprecher and Getsinger, 2000 and Wittmann, et al., 2010). Water intakes, dam locks, water gauging stations, drainage pipes, irrigation systems and
even fire prevention pipes can be colonized by *Dreissena bugensis* (Sprecher and Getsinger 2000).

*Dreissena bugensis* is native to Eastern Europe (Zhulidov et al. 2010) but spread to North America by 1989 (USGS, 2011). After initially colonizing the Great Lakes by the 1990’s (USGS, 2011), they continued to spread to the West and were found in Lake Mead in 2007 (McMahon, 2011). *Dreissena bugensis* quickly spread through the entire lake within two years of being discovered (Wittmann et al. 2010 and Hickey 2010). *Dreissena bugensis* growth patterns and life history characteristics within this new environment are not fully understood because their invasion into Lake Mead is relatively recent.

Understanding *Dreissena bugensis* growth rates in laboratory studies can shed light on how they grow, reproduce and spread in Lake Mead. Quantifying growth rates will help us begin to understand limiting factors and energetics within this dynamic ecosystem. Reproduction is partially a function of their growth rates and thus affects the population distribution (MacIsaac, 1994 and Arendt, 1997). Growth is a dynamic process and can be affected by environmental conditions (Arendt, 1997). Understanding how growth rates relate to basin characteristics can help resource managers determine priorities for control methods. In the first section of this thesis, I conducted growth experiments with the primary goal of determining if there was a difference in growth rates between mussels from two different basins. Additionally, I characterized several important water quality parameters to identify basin differences that may influence, or be influenced by, *Dreissena bugensis* growth.
As *Dreissena bugensis* spread through Lake Mead and throughout the west, new treatments must be developed to manage their spread into water systems and sensitive environments. Currently, many types of chemical treatments are being used and developed to treat *Dreissena* populations within facilities (Claudi and Macki, 1994; Sprecher and Getsinger, 2000; Watters et al., 2012; Costa et al., 2011; Costa et al., 2012). Treatment side effects can occur both within the facility and after the treated water passes into the discharge environment. Although chlorine is a popular chemical treatment for *Dreissena*, carcinogenic byproducts, such as trihalomethanes, form when chlorine combines with organic compounds in the treated water (Claudi and Macki, 1994).

Ultraviolet (UV) may be a more appropriate treatment for facilities associated with drinking water because it has no residual (Berg, 1973) and does not have known carcinogenic byproducts. Additionally, UV may also be more efficient for certain parts of facilities, such as surfaces exposed to higher volumes of water, as UV will not dilute. In this study I tested varying intensities and total doses of UV on *Dreissena bugensis* during three life stages, veliger, juvenile and adult, and I quantified the amount of UV required for high mortality rates. Additionally, I modeled dose-response functions that relate mortality rates to UV dose. By calculating the lethal dose for 50% mortality (LD50), *Dreissena bugensis* response to UV can be compared to other species in current literature.

The subsequent chapters describe the two main studies, with Chapter 2 introducing *Dreissena bugensis* growth and the hydrology of the two Lake Mead Basins focused on in this study, Boulder Basin and Overton Arm. Chapter 3 describes the methods used to analyze differences in water quality between the two basins, as well as the methods used in *Dreissena bugensis* growth experiments. Chapter 4 describes the
results from the water quality data analysis and the *Dreissena bugensis* growth experiment and Chapter 5 synthesizes these results through discussion and conclusions. Chapter 6 begins the description of laboratory experiments used to test UV on *Dreissena bugensis* by introducing the background literature. Chapter 7 describes the methods used to test UV on *Dreissena bugensis* adults, juveniles and veligers. Since veligers were tested using short term UV exposure experiments and juvenile and adult mussels were tested using long term exposure experiments, these experiments are described separately. Chapter 8 describes the results from these two experiments, followed by Chapter 9 which synthesizes the results into a discussion and conclusion. Chapter 10 is a summary of the entire thesis and includes further research recommendations.
CHAPTER 2—DREISSENA BUGENSIS GROWTH & LAKE MEAD BASIN CHARACTERISTICS

2.1 Introduction:

Originally native to Eastern Europe (Zhulidov et al., 2010), the first discovery of *Dreissena bugensis* occurred in North America in Ontario, Canada in 1989 (USGS, 2011). By the 1990’s they had spread throughout the Great Lakes (USGS, 2011) and in early 2007 *Dreissena bugensis* was found in Lake Mead, a large, artificial reservoir located at the juncture of Arizona, Nevada and Utah (McMahon, 2011). Within two years, the population of *Dreissena bugensis* rapidly spread throughout the entire lake (Wittmann et al., 2010 and Hickey, 2010). *Dreissena bugensis* populations are expected to rapidly increase and Lake Mead carrying capacity models estimate many as $1.02 \times 10^{13}$ mussels (Cross et al., 2011).

Invasive species management is difficult because it is often not possible to predict whether an introduced species will thrive in a new environment (Mack et al., 2000). By better understanding how *Dreissena bugensis* thrive or are limited in different Lake Mead basins, we can better predict conditions that may facilitate their invasion. The invasion ecology of Lake Mead basins is important to understand because, as others have discovered, “control of biotic invasions is most effective when it employs a long-term, ecosystem-wide strategy rather than a tactical approach focused on battling individual invaders” (Mack et al., 2000). Although preventing the introduction of invasive species is more cost effective than managing established populations, understanding the specific invasive ecology of Lake Mead basins can help natural resource managers better anticipate conditions that are more or less conducive to *Dreissena bugensis* growth.
*Dreissena bugensis* growth rates affect their distribution in water bodies (MacIsaac, 1994) and likely influence population expansion rates by determining how quickly they reproduce (e.g. Arendt, 1997). Growth rates can be controlled by competition and environmental conditions (Arendt, 1997). Intrinsic to this invasive species, *Dreissena bugensis* lack sufficient North American predators (USGS, 2011) and have been shown to out-compete and replace a related invasive species, *Dreissena polymorpha*, or zebra mussels (Zhulidov et al., 2010). Baldwin et al. (2002) found that *Dreissena bugensis* can grow significantly faster than *Dreissena polymorpha*, which may provide them with a competitive advantage. Wong et al. (2012) and MacIsaac (1994) found that the smaller *Dreissena* are, the faster they grow. Studying *Dreissena bugensis* growth rates within the context of individual Lake Mead basins, contributes information about ecosystem dynamics, limiting factors and distribution patterns.

Lake Mead is important among water bodies that have been invaded by *Dreissena bugensis* because its average year-round temperature is relatively high compared to that of the Great Lakes and its anthropogenic inputs are complex from basin to basin (La Bounty and Burns, 2005). MacIsaac (1994) found that *Dreissena bugensis* growth rates varied significantly by basin when comparing two basins in Lake Erie that differed in terms of food concentration and temperature (MacIsaac, 1994). Among Lake Mead’s four main basins, *Dreissena bugensis* growth rates (increase in size/time) are important for several reasons. The ecology and environmental conditions available to *Dreissena bugensis* in Lake Mead vary from basin to basin. Additionally, watersheds contributing to each basin vary in geology, land use and anthropogenic pollutants (Rosen and Van Metre, 2010). When invasive species become established in a new ecosystem, their physiology
can disrupt and interact with that system’s dominant properties (Mack et al. 2000). Nutrient cycling is a dominant ecological property of Lake Mead, which can affect *Dreissena bugensis* physiology, growth rates and life history. Additional anthropogenic disturbances in ecosystems can further exacerbate invasive species problems and can add to their competitive advantage (Mack et al., 2000).

In this study, I sought to examine the relationship between *Dreissena bugensis* growth rates and Lake Mead basin water quality characteristics. To do this, I sought to compare laboratory growth rates of *Dreissena bugensis* grown in water from each respective basin and statistically analyze differences in nitrogen, phosphorus, carbon and chlorophyll a data of two Lake Mead basins, Boulder Basin and Overton Arm. By conducting laboratory growth experiments in which the physical environment was controlled for the two groups of mussels from each basin, I was able to isolate water chemistry variables’ correlation to *Dreissena bugensis* growth.

2.2 Lake Mead Basin Hydrology:

Lake Mead, the largest reservoir in the United States, is located on the border of Nevada and Arizona and was created by the Hoover Dam (Holdren and Turner, 2010). The elevation of Lake Mead’s surface is about 340 meters above sea level (Rosen et al., 2012). Based on average water level, Lake Mead’s surface area is 637 km² with a volume of 3.5000×10¹⁰ m³ (Holdren and Turner, 2010). The four basins that make up Lake Mead include Boulder Basin, Virgin Basin, Gregg Basin and Overton Arm (Holdren and Turner, 2010) (Figure 1). Each of these basins receives water characteristic of its respective watershed and does not fully mix (Holdren and Turner, 2010).
Figure 1: Lake Mead is located on the border of Nevada and Utah and includes four main basins, Boulder Basin, Virgin Basin, Overton Arm and Gregg Basin. Major inflow enters from the Colorado River and major outflow exits Hoover Dam to the Colorado River (USGS GIS data).

Lake Mead’s total watershed area is 435,000 km$^2$. The Colorado River watershed contributes water from an area of about 376,500 km$^2$ and flows into Gregg Basin (Holdren and Turner, 2010). The Virgin and Muddy rivers contribute water from a watershed with an area of about 21,400 km$^2$ and flow into Overton Arm (Holdren and Turner, 2010).
The residence time for water in Lake Mead varies with both inflow and releases from Hoover Dam, but is generally about one to three years (LaBounty and Burns, 2005). The amount of water going into and exiting Boulder Basin has been dynamic over the past few decades due to Las Vegas’s rapid urban development and expansion (Holdren and Turner, 2010). Inflow to Boulder Basin from the Las Vegas Wash (Figure 2), which drains the Las Vegas watershed has doubled since the 1980’s (Holdren and Turner, 2010).

The Southern Nevada Water Authority (SNWA) pulls 5.55X10^8 m^3 of water per year from Lake Mead to supply urban areas including Las Vegas (Holdren and Turner, 2010). Urban storm runoff and treated water return to Lake Mead via the Las Vegas Wash. In this arid environment, surface evaporation is considered to be “extremely” high and accounts for almost 10% of the inflow, over a million acre feet per year (Holdren and Turner, 2010). Conversely, direct precipitation onto Lake Mead’s surface accounts for less than 1% of the total inflow of water to the lake (Holdren and Turner, 2010). The amount of water that is released from Hoover Dam varies, but about 9X10^9 m^3 of water is annually discharged (Holdren and Turner, 2010).
Figure 2: The Las Vegas Wash, flows into Las Vegas Bay, which is a part of Boulder Basin. Anthropogenic inputs to Boulder Basin through Las Vegas Wash includes urban and industrial pollutants.

The configuration of the four main basins affects the physical hydrology of Lake Mead. Inflow from the Virgin and Muddy rivers combine in the Overton Arm and progress through the lake as underflow due to increased density (Holdren and Turner, 2010). Downstream of Gregg Basin and Overton Arm, Boulder Basin eventually receives those waters along with water from Las Vegas Wash (LaBounty and Burns, 2005). The physical dynamics in which Las Vegas Wash water enters Lake Mead varies seasonally, with underflow occurring in winter, interflow in summer/fall and overflow occurring in spring. Water exits Lake Mead through Hoover Dam (Figure 3), through two outlets, one
of which is at the bottom of the epilimnion, while the other is located within the hypolimnion (Holdren and Turner, 2010).

Figure 3: Water flows out of Boulder Basin through the Hoover Dam

Lake Mead’s physical characteristics and dynamics affect its water quality. Incoming waters that carry nutrients into Lake Mead generally flow into the hypolimnion and lower part of the epilimnion with limited mixing; nutrients are less available to algae at the surface in the epilimnion (Holdren and Turner, 2010). Although Overton Arm and Boulder Basin are considered oligotrophic to mesotrophic, higher nutrient levels near inlets to the lake and those areas may be considered eutrophic to hypereutrophic (Holdren and Turner, 2010). Within Boulder Basin, chlorophyll a concentrations decrease with distance from the Las Vegas Wash, with concentrations over 100 mg/m$^3$ measured where the Las Vegas Wash enters the lake (LaBounty and Burns, 2005). Lake Mead is considered a monomictic lake, thermally stratified in the summer and mixing in the
winter (Holdren and Turner, 2010). During the warm summer months, Lake Mead has an anaerobic hypolimnion (negative heterograde oxygen profile) due to biological respiration and exhibits oxygen depletion at the thermocline (Holdren and Turner, 2010). The temperature in Boulder Basin’s epilimnion ranges from 12-27 degrees Celsius in summer and 11-14 degrees in winter, with an annual average of 20 degrees Celsius (LaBounty and Burns, 2005). Boulder Basin’s metalimnion’s average temperature is about 15 degrees Celsius (LaBounty and Burns, 2005).

In addition to the physical dynamics that affect Lake Mead’s water quality, land use also affects water quality within each basin. Unlike most reservoirs, Lake Mead’s most downstream basin, Boulder Basin, contains the highest bioavailable nutrient levels due to urban discharge from Las Vegas Wash. Las Vegas Wash is a primary source of dissolved phosphorus during dry weather due to recycled waste water (Rosen et al, 2013). Comparing Boulder Basin and Overton Arm nitrate concentrations is important because recycled waste water flows into Boulder Basin. Wastewater is commonly a significant contributor of nitrate into freshwater systems (APHA, 2005). Essential for many photosynthetic autotrophs, nitrate is also the growth limiting nutrient for some (APHA, 2005). Nitrate is consumed by algae, the main food source for Dreissena bugensis, and facilitates high growth rates compared to other species of nitrogen (Wetzel, 2001). The Virgin and Muddy river watersheds are currently undergoing land use changes in which development is reducing the amount of forest and rangeland in the area (Holdren and Turner, 2010). This is affecting the water quality and quantity in Overton Arm.

At the surface of Boulder Basin, average total organic carbon concentrations (TOC) are about 0.19 mg/L (LaBounty and Burns, 2005). Much of the orthophosphorus
enters Lake Mead through the Las Vegas Wash from urban areas and treated wastewater (LaBounty & Burns, 2005). Lake Mead is phosphorus limited (LaBounty and Burns, 2005; Holdren and Turner, 2010) but large concentrations of orthophosphorus flow into Boulder Basin through Las Vegas Wash (LaBounty and Burns, 2005).

Lake Mead has accumulated a large amount of sediment since its impoundment, mostly prior to the construction of the Glen Canyon dam in 1966 (Holdren and Turner, 2010). Water flowing into Lake Mead brings heavy sediment and higher nutrient concentrations than receiving waters (Rosen et al., 2012). Analysis of sediment cores from Overton Arm provide evidence that sedimentation rates were higher during atomic testing in the 1950’s due to increased dust falling onto Lake Mead and the Muddy River/Virgin River watersheds (Rosen and Metre, 2010).

Anthropogenic pollutants within sediments in Lake Mead have been found to vary from basin to basin, with the highest levels of contaminants found in Las Vegas Bay, which is part of Boulder Basin; lower levels of contaminants have been found in Overton Arm (Rosen and Van Metre, 2010). While Las Vegas Bay receives inputs from urban and industrial areas, Overton Arm has received sediment containing isotopes possibly resulting from nuclear testing fallout over the Virgin and Muddy river watersheds (Rosen et al., 2010).

Anthropogenic inputs to the Overton Arm and Boulder Basin watersheds vary due to land use and landscape alterations. Urban expansion and industry influence the Las Vegas Wash inputs into Boulder Basin, while agriculture, nuclear testing and water diversions have influenced the watersheds feeding Overton Arm.
The Muddy River and the Virgin River flow into Overton Arm. The Muddy River contains thermal springs and the water cools as it flows downstream (Scoppettone, 1998). The Muddy River has a high mineral content, with agricultural land use increasing salt concentrations. Diversions, channelization, ditches and dams along the Muddy River have altered its natural course (Scoppettone, 1998; Rosen et al., 2012).

The Virgin River has two main forks, the North fork and East fork. The Virgin River watershed has mixed land use, consisting of undisturbed lands, agricultural areas and some urban development (Boyle and Strand, 2003). Both forks of the Virgin River experience highly erosive, frequent and intense flooding (Boyle and Strand, 2003). Snowmelt and groundwater contribute to the flow of the North Fork, while mainly rainwater contributes to the East Fork (Boyle and Strand, 2003).

The Lower Virgin River is surrounded by mountains and its watershed also consists of mixed land use (Beck and Wilson, 2003; Boyle and Strand, 2003). Groundwater interchange, perennial and ephemeral streams contribute to its flow (Beck and Wilson, 2003). Water is diverted for municipal uses and agriculture (Beck and Wilson, 2003). Overton Arm sediments contain nuclear fallout, a result of wind carrying dust from the Nevada nuclear test site. Some of the highest levels of nuclear fallout reported occurred near the headwaters of the Virgin and Muddy rivers (Rosen and Van Metre, 2010).

Urban development and historical chemical manufacturing dominate the anthropogenic influences to Boulder Basin through the Las Vegas Wash. The Basic Management Incorporated (BMI) Complex manufactured chemicals including the banned
insecticide, dichlorodiphenyltrichloroethane (DDT), near the Las Vegas Wash for about 30 years from the 1940’s to the 1970’s (Rosen and Van Metre, 2010). Waste was pumped into unlined ponds, which allowed chemicals to contaminate Las Vegas Wash, evident in sediment, fish and water samples (Rosen and Van Metre, 2010). Additionally, a manganese mine also operated within the Las Vegas Wash watershed (Rosen and Van Metre, 2010). Las Vegas Bay contains higher levels of manganese and arsenic than Overton Arm. Polychlorinated biphenyls (PCBs) can be found in sediments in Las Vegas Bay sediments, but have not been detected in Overton Arm sediments (Rosen and Van Metre, 2010). Las Vegas Bay sediments contain higher levels of lead, polycyclic aromatic hydrocarbons (PAHs), DDE, a byproduct of DDT and tetrachlorodibenzo-p-dioxin (TCDD) compared to Overton Arm and Virgin Basin (Rosen and Van Metre, 2010).

2.3 Dreissena Bugensis Growth Rates

As the hydrology and water characteristics of each Lake Mead basin vary, the Dreissena bugensis invasion patterns also likely vary and are unique to each basin. Measuring growth rates of Dreissena bugensis from each basin is expected to shed some light on how the physiology of Dreissena bugensis responds to different conditions.

Dreissena polymorpha and Dreissena bugensis can clarify water by removing, not only phytoplankton, but other planktonic animals, chemicals, nutrients, metals and suspended sediment from the water column and depositing these materials in feces and pseudofeces. Additional information is needed to determine how these inputs affect Dreissena bugensis growth and population dispersion. Determining how inputs of nutrients, such as nitrogen and phosphorus, influence Dreissena bugensis growth is
important to water resources management since recycled wastewater containing these nutrients flows directly into Lake Mead. Iron, zinc, manganese, copper, cobalt, molybdenum, and nickel are important metals to aquatic life (Sunda and Huntsman, 1998; Watanabe, 1997) while other metals such as cadmium, mercury, lead, tin and chromium are toxic in high concentrations because they replace nutrient metals at metabolic sites, affecting growth rates (Sunda and Huntsman, 1998). Therefore anthropogenic inputs into Lake Mead are likely to affect *Dreissena bugensis* growth rates.

In order to examine the life history characteristics and growth rates of *Dreissena bugensis* from two different basins, I conducted growth experiments in September, October and November of 2011 and March of 2012. These experiments were done under controlled conditions in the laboratory. The first experiment’s goal was to compare growth rates of mussels from two different locations within a single basin to determine if growth rates varied within that basin. The second growth experiment’s goal was to compare growth rates of mussels from two different basins—Boulder Basin and Overton Arm.
CHAPTER 3—METHODS

3.1 Mussel Collection:

Mussels were collected from Lake Mead Marina (36°01’29.51” N, 114°46’21.06” W), Callville Bay (36°08’55.47” N, 114°42’52.46” W) which are both within Boulder Basin and Echo Bay (36°18’10.45” N, 114°25’10.49” W), in Overton Arm. Mussels were collected in November 2011 and April 2012 (Figure 4). For each experiment, mussels were collected from Lake Mead by using a spackle knife to separate them from pier flaps. The mussels were rinsed in lake water then placed in ventilated containers filled with Lake Mead water and immediately transported to the laboratory. At the laboratory, the mussels were rinsed with deionized water to remove large pieces of algae, plankton and detritus. Dead mussels were identified and discarded. The experimental mussels were then placed in aerated aquariums to acclimate to laboratory conditions, for 24 to 72 hours. Mussels were kept separate according to collection site. Growth experiments were run immediately after acclimation and all previously collected mussels were removed from acclimation tanks before new mussels were brought to the laboratory.
Figure 4: Lake Mead sampling locations for *Dreissena bugensis* growth experiments including Callville Bay, Lake Mead Marina and Echo Bay.

3.2 Water Collection & Storage:

Mussels were cultured in water specific to their collection site (Lake Mead Marina, Callville Bay and Echo Bay) (Figure 5 (a)). Water was collected and transported immediately to the laboratory where it was filtered using a 35 μm mesh filter to remove plankton, large pieces of algae and sediment; water was stored in aerated, lightly covered five gallon buckets (Figure 5 (b)).
3.3 Mussel Acclimation:

Prior to experimentation, mussels were acclimated to laboratory conditions in aerated aquariums that were held at room temperature (Figure 6). Aquariums were aerated and fitted with sponge filters and aquariums lights (Figure 6). Aquarium lights were timed for 12 hours of light and 12 hours of darkness to maintain normal algae growth in the tanks; a layer of bioballs at the top of each tank added surface area for bacteria growth. During acclimation periods, mussels were not administered additional food, other than the algae present in filtered Lake Mead water. Filtered Lake Mead water was used for all aquariums and experimental containers. During acclimation periods, 25% of the water from each aquarium was exchanged, daily, with fresh, filtered Lake Mead water.
Mead water. Pseudofeces were removed during water exchanges through siphoning to reduce the potential for re-suspension.

Figure 6: Acclimation aquariums used to acclimate mussels to laboratory conditions in preparation for growth experiments.

3.4 Experimental Design & Set-Up:

After selecting and measuring live mussels from the acclimation aquariums, groups of six similarly sized mussels were placed in 1.5 L lidded, aerated plastic containers, containing 1 liter of filtered Lake Mead water. The mussels were kept in an incubator (VWR Signature Diurnal Growth Chamber, Model 2015) (Figure 7) at 20 degrees Celsius. The incubator lights remained off during experiments to avoid uncontrolled amounts of algae growth in the experimental containers and to prevent interference with natural Lake Mead food condition. Air was introduced through a 0.2 μm with Gelman Acro50 air filter to help the natural seston remain in suspension. Filtered Lake Mead water was replaced on 1-3 day intervals to maintain adequate food supply.
Figure 7: Incubator contained experimental buckets to control temperature for *Dreissena bugensis* growth experiments. Experimental buckets were gently aerated with filtered air through tubes.

3.5 Algae Culturing:

Nannochloris algae was cultured in the laboratory and used to supplement filtered lake water for some groups of mussels (Figure 8 (a)). Nannochloris spp., is a green, round algae with a cell diameter of approximately 1 - 3μm; Nannochloris grows with small to minimal clumping. To culture Nannochloris, a ratio of 0.0004 AquaFarms AlgaeGro© to 1,000 mL of nanopure/Milli-Q water was used. The media (water and AlgaeGro) was then autoclaved for 30 minutes at 120 degrees for getting rid of unwanted bacteria. After cooling, it was ensured that the media’s pH was between 7.5 and 8 before adding Nannochloris algae seed for culture.
Figure 8: Left (a): Nannochloris algae cultured in the laboratory was used in growth experiments and administered to mussels to examine the maximum growth of *Dreissena bugensis*. Maximum *Dreissena bugensis* growth rates were compared to *Dreissena bugensis* administered only filtered Lake Mead water only. Right (b): Nannochloris algae being pipetted into centrifuge tubes to be able to administer a consistent and known amount of algae to each container within the maximum growth groups.

The algae was placed on a table grow lamp until sufficient growth had occurred. An Eppendorf Repeater® Plus automatic pipette was used to transfer 15 mL of the algae into centrifuge tubes which was then centrifuged for 20 minutes at 4,000 revolutions per minute to separate water from the media (Figure 8 (b)). The water was then replaced, using the automatic pipette, with 15 mL of filtered Lake Mead water to bring the algae back into suspension. An automatic pipette was used to ensure that the exact same amounts of algae were used during all experiments. The algae was re-suspended by hand using a disposable pipette.

A Shimadzu UV-1700 spectrophotometer was used to determine the algae density with wavelengths 664.0 and 750.0 nm (Figures 9 and 10). A regression curve of absorbance, at each wavelength (664.0 and 750.0), and cell density in cell/mm³, was used
to determine the amount of the supplemental Nannochloris administered to the mussels.

These curves were developed by and are currently used in the Acharya Laboratory. Algae was frozen for storage and defrosted to room temperature before administering to the mussels.

![Graph showing absorbance at 664 nm versus cell concentration.](image)

**Figure 9:** Nannochloris algae absorbance at 664 nm using the Shimadzu UV-1700 spectrophotometer was correlated to cell density determined by microscopy. The equation was used to calculate the amount of algae cells administered to mussels in growth experiments to examine maximum growth.
Figure 10: Nannochloris algae absorbance at 750 nm using the Shimadzu UV-1700 spectrophotometer was correlated to cell density determined by microscopy. The equation was used to calculate the amount of algae cells administered to mussels in growth experiments to examine maximum growth.

3.6 Growth Measurements:

Through trial experiments, it was determined that growth must be measured by mass rather than length or width. Trial experiments attempting to measure shell length and width agreed with MacIsaac (1994) showing that shell width and length can decrease even as body mass increases in *Dreissena*. Methods to measure mussel size for growth experiments vary in existing literature, for example, MacIsaac (1994) used dry mass while Baldwin (2002) used wet mass. For the first experiment, dry mass of the mussel was measured and the shell mass was excluded to eliminate error due to potential shell degrowth as observed in trial experiments and by MacIsaac (1994). The first experiment
compared growth of *Dreissena bugensis* from two locations within Boulder Basin, Lake Mead Marina and Callville Bay.

To obtain the dry weight mussel body only, the shell was removed by dissecting the mussel using a scalpel starting from the pedal gape (which allows extrusion of the foot and large byssus) and cutting the mussel open between the two shells at an angle so that the body was separated to one side. Next, the body was scraped from the shell and placed on a pre-weighed drying tin (Figure 11) and dried at 60 degrees Celsius for 48 hours. The dry weight was then measured.

Methods were changed to follow Baldwin (2002) for the second experiment because complete removal and transfer of tissue could not be achieved consistently. For the second experiment, a tissue was used to blot the mussels dry and the entire mussel, including the shell was weighed, as described by Baldwin (2002). At the beginning of the experiment, five mussels were measured for length and width, and then dissected to be dried and weighed. After each week had passed for the following five weeks, one mussel was removed from each group’s container and measured. Somatic growth rates were calculated based using the following formula (Acharya, et al. 2006; MacIsaac, 1994 and Baldwin et al., 2001):

\[
\mu = \ln \left( \frac{\text{final weight}}{\text{initial weight}} \right) / \text{week}
\]
To determine if there were differences in growth rates between basins, it was first necessary to determine if there were differences in growth rates within a single basin. A growth experiment was conducted to compare *Dreissena bugensis* growth rates from Lake Mead Marina (36°01'31.83"N 114°46'16.4W) and Callville Bay (36°08'22"N 114°42'55.63W). Mussels and Lake Mead water were taken from each location following the protocol described above. In this experiment, 36 mussels from each location were selected and separated into groups of six per container. An additional group of 36 mussels were selected from Boulder Basin and were administered supplemental Nannochloris algae to represent the maximum potential growth. The purpose of the maximum growth group was to be able to compare growth rates of mussels in water from each basin with the maximum potential growth rates measured from algal supplemented mussels. Mussels with lengths between 8.0 mm and 8.99 mm were selected to ensure that the range in size remained below 1 mm. Over the course of six weeks, one mussel from
each container was removed each week, dissected, dried and weighed to determine the growth rate i.e., change in body mass over time.

For the final experiment, mussels with lengths between 7.00 and 7.99 mm were selected and separated into groups of six mussels per container. Six replicate containers were set up to represent each group: Boulder Basin, Overton Arm and the “maximum growth” group. Over the course of six weeks, one mussel from each container was removed for measurement. Length, width and wet weight measurements of entire mussels (shell and body) were taken during this experiment. To measure wet weight, five mussels were removed at a time from the water and each was blotted dry with a Kim wipe and allowed to drain for approximately ten minutes before weighing.

3.7 Data Analysis:

Statistical analysis was performed to determine differences between basins in chlorophyll a, total organic carbon, nitrate, orthophosphate and *Dreissena bugensis* growth rates. Statistical analysis was performed using JMP 5.0.1 (SAS Institute Inc.) software. ANOVA for normally distributed data sets and the Wilcoxon Test and Kruskall-Wallace (Rank Sums), for non-normally distributed data sets were used to determine the significance of difference. A 95% confidence interval (p value of 0.05) was used for all statistical analysis. In figures with histograms, statistical difference is indicated by different letters. For data sets that contained values below the detectable limit, the non-parametric Kruskall-Wallace (Rank Sums) was used to determine differences because this method treats the non-detectable values as “ties” and is less sensitive to error than parametric tests (USEPA, 1989).
Descriptive statistics were calculated using excel. For chlorophyll a, the sample size was 32, with a mean value of 0.56 mg/m$^3$, for orthophosphate the sample size was 334, with a mean value of 1.3 μg/l, with a standard deviation of 0.84. The sample size for total nitrate was 76, with a mean value of 0.52 mg/l and a standard deviation of 0.18. For total organic carbon, the sample size was 204 with a mean of 2.7 mg/l and a standard deviation of 0.34. For the growth experiments, six containers were created for each condition, with six mussels in each container, for a sample size of 18. The mean somatic growth rate was 0.011/day with a standard deviation of 0.0049.

3.8 Lake Mead Water Quality Characterization:

Overton Arm and Boulder Basin water characteristics were compared to characterize the differences in chlorophyll a, total nitrate, orthophosphate and total organic carbon (TOC). Nitrogen, phosphorus and carbon are the essential “building blocks” of cells and are important in aquatic ecosystems. Chlorophyll a and total organic carbon were analyzed because *Dreissena bugensis* are filter feeders and consume algae and seston; therefore they are important to growth. Phosphate usually limits productivity in lakes because it exists in relatively small proportions compared to the other nutritional and structural components of life (Wetzel, 2002). In this study, orthophosphate was analyzed because it is the most significant form of inorganic soluble phosphate. Orthophosphate concentrations are usually very low, usually making up less than 5% of total phosphorus (Wetzel, 2002). Anthropogenic inputs, such as those from recycled waste water and urban storm water runoff can contribute orthophosphate to lakes. In this study nitrate was analyzed because nitrate is an essential nutrient to algae and all aquatic plant life.
Water quality data from the Southern Nevada Water Authority’s Lower Colorado Water Quality Database was analyzed. Lake Mead is one of the most intensely sampled water bodies in the United States (Rosen et al., 2012). Permission to access this database was provided by Susan Holmes and Peggy Roefer, through SNWA’s Lower Colorado River Regional Water Quality Database Interest form system. Since the growth experiment comparing growth rates for Overton Arm and Boulder Basin was conducted in the springtime, only March, April and May water quality data was used. Data from sampling sites, “VR13.0—Overton Arm near Big Horn Islands” (36°17′28.78″N 114°23′11.40′ W) and “BB_7—Boulder Basin – west of Boulder Island on SW tip of largest island”, (36° 2′44.80 N 114°46′52.46′ W) were selected because they contained the most comparable and recent data sets and were the closest, geographically, to the sites where water and mussels were taken from for this experiment. Water and mussels were collected from the Lake Mead Marina site in Boulder Basin and Echo Bay (36°18′12.95″N 114°24′54.88W) in Overton Arm. Total organic carbon was compared between Overton Arm (site number VR_13) and the Boulder Basin (BB_3) site located at 36°04′14.57″N 114°46′54″ because TOC data was not available for the BB_7 site. Sampling details are listed in Appendix 2.
CHAPTER 4—RESULTS

4.1 Growth Experiments:

Mussels were successfully grown in the laboratory using the methods outlined above. All experiments showed positive growth rates in the experiments. Growth rates of mussels from two locations within Boulder Basin, Lake Mead Marina and Callville Bay were not significantly different. When compared to the maximum potential growth rate, however, both groups of mussels grown in Boulder Basin water grew significantly slower than the algal supplemented mussels.

![Graph showing somatic growth rates](image-url)

Figure 12: Somatic growth rates of mussels from two sampling locations within Boulder Basin, Lake Mead Marina and Callville Bay and mussels given supplemental food, “Maximum Growth”. There was no significant difference in growth rates in mussels from the two locations within Boulder Basin, but the maximum growth rate was significantly
higher. Letter above bars represent pairwise comparison (Wilcoxon) test; error bars are standard error of mean.

Somatic growth rates of mussels from Boulder Basin and Overton Arm were significantly different. The growth rate (wet body weight) of mussels from Overton Arm was significantly higher than the growth rate of mussels from Boulder Basin. The mussels receiving supplemental food, the “maximum growth” group grew at a faster rate than the mussels in filtered Lake Mead water only. The difference in growth rates between mussels from Overton Arm and the maximum growth rate were not significantly different (Figure 18).

![Bar graph showing growth rates of mussels from Boulder Basin, Echo Bay, and a Maximum Growth group.](image)

**Figure 13:** Growth rates of mussels with water from Boulder Basin, Echo Bay and a Maximum Growth group (administered additional algae). Based on Wilcoxon-Kruskal-Wallace, mussels from Overton Arm grew significantly faster than mussels from Boulder Basin.
Basin under laboratory conditions. Error bars are standard error of mean. Different letters indicate above bars indicate significant differences.

4.2 Lake Mead Water Quality Characterization:

Water quality was compared between Overton Arm and Boulder Basin, which can help to explain the results from the *Dreissena bugensis* experiments comparing growth rates of mussels from each basin. Results showed that there were significant differences in chlorophyll a, total organic carbon, nitrate and orthophosphate when comparing the two basins. The results from water quality data analysis showed that the total nitrate and orthophosphate during spring months were both significantly higher in Boulder Basin, with $p < 0.0001$ for both total nitrate and orthophosphate. Average total nitrate was 0.66 mg/L for Boulder Basin and 0.31 mg/L for Overton Arm, (Figure 12). Although average orthophosphate concentrations were significantly different between the two basins, both basins had very low concentrations with respect to levels needed for productivity (Wetzel, 2001). Concentrations were 1.83 $\mu$g/l and 1.19 $\mu$g/l for Boulder Basin and Overton Arm, respectively (Figure 12).
Figure 14: Average total nitrate was significantly higher in Boulder Basin compared to Overton Arm (p < 0.0001). Error bars are standard error of mean. Different letters above bars indicate significant differences.

Figure 15: Average Orthophosphate concentrations during spring months (March, April, May) 2007 through 2010. Orthophosphate concentrations were higher in Boulder Basin than Overton Arm. Error bars are standard error of mean. Different letters above bars indicate significant differences (Kruskal-Wallace, Rank Sums); (p < 0.0001).
Overton Arm had significantly higher average chlorophyll a than Boulder Basin (p=0.032). Average chlorophyll a for Boulder Basin was 0.50 mg/m$^3$ and 0.64 mg/m$^3$ in Overton Arm (Figure 14).

![Bar chart showing Chlorophyll a (mg/m$^3$) for Boulder Basin and Overton Arm](image)

Figure 16: Average spring (March, April, May) Chlorophyll a during years 2008-2011. Overton Arm contained significantly higher levels of Chlorophyll a than Boulder Basin. Letters above bars indicate statistical difference by Kruskal-Wallis (Rank Sums) (p=0.032); error bars are standard error of mean.

Total organic carbon (TOC) in Boulder Basin was significantly higher with a value of 2.33 mg/L compared to Overton Arm, which had a value of 2.80 mg/L (Figure 15).
Figure 17: Overton Arm had significantly higher Total Organic Carbon (TOC) compared to Boulder Basin during spring months, March, April and May between 2005 to 2012. Error bars are standard error of mean and letters above bars indicate statistical difference. To represent Boulder Basin, site “BB_3” was used here, rather than “BB_7”.
CHAPTER 5—DISCUSSION

Overton Arm conditions yielded significantly higher *Dreissena bugensis* growth rates compared to Boulder Basin. Significantly higher chlorophyll a and total organic carbon concentrations were found in Overton Arm, which could reflect higher quantities of food available to the mussels. Overton Arm *Dreissena bugensis* growth rates were not significantly lower than maximum growth rates, which means that *Dreissena bugensis* growth rates are reaching their potential in Overton Arm. Conversely, Boulder Basin growth rates were significantly lower than the maximum potential growth rates. This means that Boulder Basin *Dreissena bugensis* are likely not growing at their highest potential.

During lake-wide surveys, the highest densities of *Dreissena bugensis* were found in Boulder Basin and found evidence that growth rates were lower in Boulder Basin compared to Overton Arm when examining shell lengths in samples over time (Wittmann et al. 2010). Wittmann et al. (2010) suggested that *Dreissena bugensis* may be limited by food resources. This may be reflected in the lower chlorophyll a and total organic carbon accompanied with lower growth rates in Boulder Basin. Wittmann et al. (2011) found the highest densities of *Dreissena bugensis* in Boulder Basin and suggested that the population was, “exhibiting density dependence, reflecting competition for food and /or habitat that is limiting to individual mussel growth as well as population expansion.” The results presented in this study agree.

Although nutrient levels were generally higher in Boulder Basin compared to Overton Arm, the food quantity may be lower in Boulder Basin. If chlorophyll a concentrations reflect food quantity, which was found to be lower in Boulder Basin, this
may mean that mussels may grow slower with lower algae concentrations. If additional food became available to *Dreissena bugensis* in Boulder Basin, increased growth rates could follow suit.

Similarly to this study, different growth rates by basin were also found in MacIsaac (1994) who studied *Dreissena* in two basins in Lake Erie. MacIsaac studied both in-situ and laboratory growth rates, and attributed much of the difference in *Dreissena* growth rates to lake physical characteristics. Food concentration, water flow rates and temperature may have caused the difference in growth rates among basins in Lake Erie (MacIsaac, 1994). By isolating variables to water characteristics, and eliminating physical conditions such as temperature and water flow rates, differences in water alone have been shown here to have an effect on *Dreissena bugensis* growth rates.

When examining growth rates and orthophosphate, Boulder Basin had higher orthophosphate levels and lower *Dreissena bugensis* growth rates. Both basins had very low levels of orthophosphate. In lakes invaded by *Dreissena*, the pre-invasion phosphorus concentrations make a difference in how *Dreissena* end up altering trophic interactions (Sarnelle et al., 2012; Qualls et al., 2007). The presence of *Dreissena* in waters with pre-invasion low phosphorus conditions have been found to correlate with higher frequency of cyanobacteria blooms than lakes with high phosphorus pre-invasion conditions (Conroy et al., 2005; Quall et al., 2007; Sarnelle et al., 2012). Mussels can increase the concentration of nutrients available to algae by transforming them from those adsorbed to particulates, which have settled, and redistributing them back into the water column in dissolved form. This process leads higher concentrations of bioavailable phosphorus, resulting in cyanobacteria blooms (Beaver et al. 2010 and Higgins and
Vander Zanden, 2010). Most phosphorus entering Lake Mead (15,600 kg/d) is bound to sediment (Rosen et al., 2012). Additionally, *Dreissena* have been found to recycle nutrients faster than native zooplankton, which increases the availability of nitrogen and phosphorus to cyanobacteria (Conroy et al., 2005). Many forms of cyanobacteria are not consumed by quagga mussels.

*Dreissena* have relatively low phosphorus requirements and high growth rates compared to many native species (Morehouse et al., 2013), which can contribute to their invasion success, especially in oligotrophic conditions. Morehouse et al. (2013) found that excessively high phosphorus levels actually limited *Dreissena* growth. Based on stoichiometry research (Morehouse et al. 2013), the ratios of carbon to phosphorus is likely more important than the actual concentrations of carbon available. Morehouse et al. (2013) found that the interactions between nitrogen and *Dreissena* invasion are complex. Additionally, as Holdren and Turner (2010) found higher levels of nutrients at the inlets of Lake Mead, the growth rates and population dynamics in those areas are likely affected.

*Dreissena bugensis* are very efficient in terms of food consumption and growth, with some studies resulting in *Dreissena bugensis* growth rates 20 times higher than *Dreissena polymorpha* (Baldwin et al., 2002). Invasive *Dreissena* have a competitive advantage in low phosphorus waters; additionally, *Dreissena* can also facilitate cyanobacteria blooms in low phosphorus waters. Therefore, areas with low concentrations of phosphorus should be prioritized over areas experiencing eutrophication for *Dreissena* management because these areas may be impacted more than areas with higher phosphorus levels. In this study, while Boulder Basin was found to
have higher nitrogen and phosphorus concentrations, *Dreissena bugensis* were found to grow faster in Overton Arm water conditions. Areas with lower, rather than higher nutrient levels, may be priority areas for *Dreissena bugensis* control and management in terms of ecosystem damage control. Additionally, since Overton Arm has higher concentrations of chlorophyll a, with very low phosphorus, it is possible that the presence of *Dreissena bugensis* can be facilitating algae growth if algae growth is dominated by cyanobacteria.

Since *Dreissena bugensis* consume algae as food, it makes sense that the basin that contains higher chlorophyll a concentrations would yield mussels with higher growth rates. Additionally, the simultaneous occurrence of high chlorophyll a and high *Dreissena bugensis* growth rates may mean that the presence of *Dreissena bugensis* may increase the occurrence of algae.

This study provides evidence that *Dreissena bugensis* growth rates are lower in Boulder Basin compared to Overton Arm; one of the most dramatic differences between these two basins is the anthropogenic influence of the Las Vegas Wash and the input of various toxins into Boulder Basin. When studying *Dreissena bugensis* population density and distribution in Lake Mead, Wittmann et al. (2010) suggested that toxins from Las Vegas Wash may account for the relatively low number of juvenile and adult *Dreissena bugensis* in Las Vegas Bay. Wittmann et al. (2010) found that population density and distribution was markedly different from basin to basin. Wittmann et al. (2010) found the biggest differences in densities when comparing densities of Las Vegas Bay to the rest of Boulder Basin and Overton Arm. Although Wittmann et al. (2010) found that the number of veligers in Las Vegas Bay was relatively high, there were low numbers of adults. In
this study, mussels from Callville Bay had higher, but not significantly higher, growth rates than mussels from Lake Mead Marina. Lake Mead Marina is downstream of the Las Vegas Wash, while Callville Bay is upstream of the Las Vegas Wash. Pollutants from Las Vegas Wash may limit *Dreissena bugensis* growth rates.

Somatic growth rates of Lake Mead *Dreissena bugensis* in this study are lower than previously published. Baldwin et al. (2002) compared *Dreissena bugensis* growth rates based on food type and, in general, found higher growth rates than this study, but also held the animals in warmer temperatures and provided a higher concentration of food. Low food quality may affect *Dreissena bugensis* energy expenditures and growth rates (Beaver et al. 2010). Lake Mead may have lower food quality than other lakes, which could also explain the lower growth rates found in this study compared to Baldwin et al. (2002). MacIsaac (1992) cited evidence that reproduction may weaken the link between water temperature and growth rates. Lake Mead’s relatively high temperatures mean that Lake Mead *Dreissena bugensis* spawn at least twice per year (Wittmann et al. 2010). The energy required for additional spawning compared to *Dreissena bugensis* in other locations, such as the Great Lakes, may lead to slower growth rates, such as those found by Baldwin et al. (2002). Additional research should be conducted to examine how physical conditions in Lake Mead affect *Dreissena bugensis* growth rates.

*Dreissena bugensis* growth rates likely vary by size, as MacIsaac (1994) found that in certain areas, small mussels (5 mm) grew significantly faster than larger mussels (15 mm). Additional research should be conducted to determine the growth rates of *Dreissena bugensis* at various life stages.
CHAPTER 6—ULTRAVIOLET RADIATION AS A POTENTIAL TREATMENT FOR
DREISSENA BUGENSIS

6.1 Introduction:

Ultraviolet (UV) radiation has potential as a non-chemical treatment for quagga mussels (Dreissena bugensis). The UV portion of the electromagnetic spectrum consists of the wavelengths from 100 to 400 nm within the electromagnetic spectrum and is subdivided into three main groups, UV-A, UV-B and UV-C consisting of wavelengths from 400 to 315 nm, 315 to 280 nm and 280 to 100 nm, respectively (Serway and Jewett, 2008). UV is known to be biologically damaging, and limited evidence exists that it can kill Dreissena. To develop UV as a part of an engineering solution to treat Dreissena bugensis, the lethal amount of UV needed for Dreissena bugensis at various life stages must be quantified. As they swiftly multiply, remove algae and cover surfaces, Dreissena bugensis cause ecological destruction, and economic damage in sectors including water treatment, power generation, industry and agriculture (Sprecher and Getsinger, 2000 and Wittmann, et al., 2010). Solutions are needed to manage Dreissena invasions in water intakes, locks, gauging stations, drainage pipes, irrigation systems and even fire prevention pipes (Sprecher and Getsinger, 2000). UV may be developed to treat Dreissena in these areas.

Many types of chemical treatments are being developed and used to manage Dreissena populations in facilities (Claudi and Macki, 1994; Sprecher and Getsinger, 2000; Watters et al., 2012; Costa et al., 2011; Costa et al., 2012). The two main types of chemical treatments are oxidants and nonoxidizing molluscicides (Sprecher and Getsinger, 2000). Oxidants include chlorine dioxide, chlorine gas, ozone, hydrogen
peroxide, bromine and permanganates (Sprecher and Getsinger, 2000), while nonoxidizing molluscicides include potassium, aromatic hydrocarbons, organic salts, metals such as copper ions, and endothall, an herbicidal compound. Treatment goals in managing *Dreissena* invasions include removing existing mussel colonies, reducing veliger settlement and eliminating reproduction. Simultaneously, consideration must be given to the treatment side-effects, both within the facility, and after the chemical has passed through the facility (Sprecher and Getsinger, 2000). Although the byproducts of oxidants are generally well understood (Sprecher and Getsinger, 2000), byproducts from chemical treatments are still a major concern. Chlorine is a popular treatment, carcinogenic byproducts, trihalomethanes, form when chlorine combines with organic compounds (Claudi and Macki, 1994).

UV may provide a treatment that has fewer side-effects than chemical treatments, such as residual chemical in discharge, making it more appropriate for certain types of facilities, such as those associated with drinking water. UV may also be more efficient than chemical treatments for certain parts of facilities, such as surfaces exposed to higher volumes of water as UV will not dilute. UV would likely produce fewer byproducts that would not increase with increased intensity levels; additionally, temperature is unlikely to affect how much UV would be needed because temperature does not affect radiation.

Limited information exists on using UV to kill *Dreissena* but some facilities have tested it and reported veliger reductions of 85% (Quagga and Zebra Mussel Control Strategies Workshop, 2008). Chalker-Scott et al. (1993), Chalker-Scott et al. (1994) and Chalker-Scott & Scott (1998) conducted limited research on *Dreissena bugensis*, quagga mussels, and *Dreissena polymorpha*, zebra mussels, and their response to UV radiation,
providing evidence that it can cause behavioral changes and mortality in adults and veligers. Quagga mussels were found to be more resistant to UV than zebra mussels. Size and age-class specific UV radiation levels needed for high rates of mortality were not specifically quantified because adult mussels of various sizes were tested together and could move to areas with different levels of UV (Chalker-Scott et al., 1993). Studies by both Chalker-Scott et al. (1993) and Wright et al. (1997) resulted in *Dreissena polymorpha* veliger mortality after UV-B exposure, with Wright et al. (1997) reporting much higher doses needed to kill the veligers than Chalker-Scott et al. (1993).

Beyond the limited number of studies examining the effects of UV on *Dreissena polymorpha* and *Dreissena bugensis* (Chalker-Scott et al., 1993; Chalker-Scott et al., 1994; Chalker-Scott et al., 1998), the effects of UV on other aquatic organisms has been studied within two main frameworks. While some UV related studies have focused on methods to combat bio-fouling (Hori, et al., 1990), most have been designed to consider the potential effects of reduced UV absorption in the ozone layer. Studies have examined UV effects on individual species such as barnacle larvae (Hori, et al., 1990), brown shrimp larvae (Wubben, 2000) and *Daphnia* (Scott et al., 1999 and Williamson et al., 2001), while other studies have looked at the variable response between species within an ecosystem context (Hurtubise et al., 1998 & McNamara and Hill, 1999).

There is considerable information about how UV affects biological systems. The primary way UV damages biological systems is through DNA damage, which has been found to occur in aquatic organisms (Palenik et al. 1990). Energy levels in UV correspond to the energy levels needed to rearrange consecutive thymine nucleotides in DNA. UV can fuse together two consecutive thymine nucleotides on a DNA strand with
a covalent bond forming a thymine dimer. This changes the strand shape affecting nearby nucleotides. The production of thymine dimers leads to programed cell death (apoptosis), immune suppression, and cancer (carcinogenesis) (Schreier et al., 2007). These processes can occur more than six hours after UV-B exposure causing a delayed response (Yarosh et. al, 2000). In natural environments some organisms simultaneously use certain wavelengths of solar radiation to repair this UV damage (Wetzel, 2001). This means that the effects of UV-B can progress from immediate damage to a fluctuation between delayed damage and repair after exposure. Delayed mortality as a result of UV exposure was observed in laboratory studies by Hori et al (1990) studying *Chthamalus* larvae and by Wright et al. (1997) studying *Dreissena*. Chalker-Scott et al. (1993) and Wright et al. (1997) found that shortly after UV exposure, *Dreissena* veligers could be stunned, but remain alive for some time.

UV damage to biological systems can be reduced by mycosporine-like amino acids (MAAs) and mitigated by photorepair. Certain amino acids, MAAs, are produced within organisms that protect the cells from the sun and have been discovered in the mussel, *mytilus galloprovincialis* (Chioccara et al., 1979; Chioccara et al., 1985). Photorepair, or photoreactivation, is a process in which visible and near UV solar radiation in the range 315 to 500 nm activate enzymes to repair UV-B damage (Damkaer and Dey, 1983). Photoreactivation has been cited to repair UV-B damage in several organisms, but how much UV-B damage can be mitigated through photoreactivation is not well understood and most likely varies among species (Damkaer and Dey, 1983).

While there is a significant body of evidence that UV-B is capable of affecting biological systems and can kill *Dreissena*, current literature lacks 1) how UV-B affects
the *Dreissena bugensis* species and 2) how much UV-B would be needed to kill *Dreissena bugensis* at different life stages. To understand if UV-B could be a viable method for treating *Dreissena bugensis* it is necessary to determine the dose and intensity levels needed. The objective of this study is to empirically model the UV-B quantity needed to kill *Dreissena bugensis* through two types of laboratory experiments, long-term and short-term experiments. Adult and juvenile *Dreissena bugensis* were continuously exposed to three levels of UV-B over the long-term until 100% mortality was attained. Veligers were exposed to short-term durations of UV-B at three set intensities, after which mortality was tracked. A second objective to this study was to determine if either total exposure time or total intensity had a larger effect on mortality, when the total dose is the same. Results from this study will contribute to the development of UV-B engineering applications for water intake pipes and other apparatus.
CHAPTER 7—METHODS

7.1 Specimen Collection and Handling:

Adult and juvenile *Dreissena bugensis* were collected from Lake Mead, near Kingman Wash (36°02′09.56″ N, 114°42′36.16″W) (Figure 19) by SCUBA diving during the fall of 2012. Veligers were collected from Callville Bay Marina, Lake Mead (36°01′29.51″ N, 114°46′21.06″) in early 2013 using a plankton net/tow (63 μm) (Wildco Design, Wildlife Supply Co.) (Figure 19).

![Lake Mead Sampling Locations](image)

Figure 18: Juvenile and adult *Dreissena bugensis* samples for UV experiments were obtained from Kingman Wash. Veliger samples used in UV experiments were obtained from Callville Bay.
Mussels were placed in small buckets filled with Lake Mead water and immediately transported to the laboratory where they were rinsed with DI water several times to remove zooplankton and large detritus. The mussels were placed in aquariums filled with filtered Lake Mead water and allowed to acclimate to laboratory conditions for 48 hours. The contents of each plankton tow were filtered in the field using a 250 μm mesh filter to remove predatory zooplankton (Figure 20). The veligers were immediately transported to the laboratory where they were placed in larger beakers.

Figure 19: Field equipment to collect veligers from Lake Mead. A plankton net was used to collect veligers from Lake Mead. Veligers were collected from Lake Mead for UV experiments.

Mussel acclimation aquariums were aerated and fitted with sponge filters. Aquarium lights, set by timers for 12 hours of light and 12 hours of darkness were used to
maintain normal algae growth in the tanks. During acclimation periods, 25% of the filtered Lake Mead water in each aquarium was replaced and pseudofeces were removed from the aquariums through siphoning to reduce re-suspension.

Mussels were removed from the acclimation tank based on their size class and placed in an intermediary container to ensure that they were actively siphoning before starting the experiment. Two size classes were selected, including juveniles of 6.4 – 9 mm in length and adults of 13 – 19 mm in length. Ten mussels were placed in each experimental container and four replicate containers were prepared for each condition to be tested.

Groups of veligers were transferred from the large beaker, containing the plankton tow collection, to a watch-glass with a disposable pipette. After a few minutes, active veligers could be identified using cross-polarization microscopy (Nikon SMZ1000 dissection microscope fitted with a cross polarization lens). The average veliger size was approximately 150 μm in diameter (sample size 16, standard deviation 32). Petri dishes were used as experimental containers to hold the veligers during UV exposure. Filtered Lake Mead water was measured in 20 mL amounts and placed in each petri dish using an automatic pipette (Eppendorf Repeater® plus model 307006Z) (Figure 21). Three active veligers were individually transferred into single petri dishes with a disposable 1.5 mL pipette. In order to maintain a consistent environment for all experimental veligers, they were stored in an incubator when they were not being examined under the microscope or exposed to UV. The incubator was set at 20 degrees Celsius, partly shaded from a partly lit lamp set for 12 hours darkness/12 hours light to represent natural light conditions. Each dish was administered about 6.3x10^4 cells of lab-cultured Nannochloris algae
similar to Wright et al. (1997) and an additional 5 mL of filtered Lake Mead water one week after exposure in order to ensure that starvation was not a factor in resulting mortality.

Figure 20: Cross polarization microscopy was used to transfer active veligers in two steps to petri dishes. First, veligers from the plankton tow were transferred to a watch glass and active veligers were distinguished from dead veligers and other animals. Second, individual veligers were transferred to petri dishes. Once in petri dishes, veligers were recounted and it was verified that they were still active before being exposed to UV.
7.2 Media:

Filtered Lake Mead water was used in both the acclimation tanks and experimental containers. Lake Mead water was transported to the laboratory and filtered using a 35 \( \mu \)m mesh filter to remove large detritus and zooplankton, while leaving natural seston in place. The ash free dry mass (g) of carbon (i.e., food) in the filtered water was measured at 0.9 mg /l. In chronic exposure experiments, temperature, ozone and ammonia levels were monitored and were relatively constant throughout the experiments. Water was changed daily during both of the chronic exposure experiments.

7.3 Experimental Design:

Adult and juvenile mussels were placed in a reusable coffee filter (10 cm in diameter) that was centered within a shallow polyethylene box (29 cm x 15cm). The coffee filter acted as a cage to concentrate the mussels in a small area so that each mussel would receive a similar amount of UV radiation, without restricting water flow. The portion of the plastic box outside of the coffee filter was covered to exclude UV radiation so that only the area containing the subject mussels was affected. Each plastic box contained 800 mL of Lake Mead Water, filling the box to a depth of 1.7 cm (Figure 21). Petri dishes filled with 20 mL of water were used to contain three veligers in each dish; four replicate dishes were prepared for each condition.
A series of initial trial experiments were conducted to determine an effective range of UV radiation to test on the mussels. In similar studies, a range of 420 and 9.2 x $10^4$ mJ/cm$^2$ dose (intensity times time) was used; the experiments presented here used a range of 360 mJ/cm$^2$ to $3 \times 10^5$ mJ/cm$^2$ UV dose. UV radiation was supplied by Ushio G8T5E 7.2 watt midrange UV fluorescent bulbs (306 nm), with length of 28.68 cm, diameter of 1.55 cm. A Model 5.7 - UV Meter Solarmeter (Solartech Inc., Harrison Twp, MI, U.S.A.) was used to measure UV intensity. Lamps were set up above the experimental containers, within a distance to each mussel cage to expose each cage to a set level of irradiance. Based on published studies and on preliminary experiments, three UV intensity levels, 0.5 mW/cm$^2$, 0.3 mW/cm$^2$ and 0.1 mW/cm$^2$, and one control.
condition, were used. Maximum U.S. daily levels of UV-B (305 nm) are around $1 \times 10^{-3}$ mW/cm$^2$ and the daily sum is about $2 \times 10^2$ mJ/cm$^2$. The adult and juvenile mussel control groups received partial aquarium lighting, which contained no UV, for 12 hours per day. The experimental design is summarized in Table 1.

Table 1: Experimental design summary for chronic and acute experiments, including observation frequency, number of replicates, number of animals per container, total number of animals tested, UV-B irradiance exposure levels and durations.

<table>
<thead>
<tr>
<th></th>
<th>Veliger</th>
<th>Juvenile</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronic Exposure</strong></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>Acute Exposure</strong></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>Intensity Levels</strong></td>
<td>0, 0.1, 0.3, 0.5 (mW/cm$^2$)</td>
<td>0, 0.1, 0.3, 0.5 (mW/cm$^2$)</td>
<td>0, 0.1, 0.3, 0.5 (mW/cm$^2$)</td>
</tr>
<tr>
<td><strong>Total Exposure Duration</strong></td>
<td>1 hr., 2 hr., 3.5 hrs.</td>
<td>72 hrs.</td>
<td>20 days</td>
</tr>
<tr>
<td><strong>Observation Frequency</strong></td>
<td>after exposure: 24 hrs., several days, 2 weeks</td>
<td>every 24 hrs.</td>
<td>every 24 hrs.</td>
</tr>
<tr>
<td><strong>Replicate Containers</strong></td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Animals Per Container</strong></td>
<td>3</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total Number of Animals</strong></td>
<td>144</td>
<td>160</td>
<td>160</td>
</tr>
</tbody>
</table>
7.4 Chronic Toxicity Tests:

Adult and juvenile mussels were tested separately in continuous exposure tests, through two different experiments. Four replicate containers with ten mussels in each container were exposed to 0.5 mW/cm², 0.3 mW/cm² and 0.1 mW/cm² until 100% mortality was reached; mortality was recorded daily.

Mortality was determined by gently poking the mussels; if the mussels responded by closing, they were deemed alive. If they did not respond within a minute or two, the shells were gently forced closed and if they opened immediately, they were deemed dead (Claudi and Macki, 1994 and Costa et al., 2011; Watters et al., 2012). Dead mussels were removed, placed in zip-lock bags and frozen for storage.

7.5 Acute Toxicity Tests:

Acute toxicity tests were run on veligers. Three experiments were conducted to test three different time durations of exposure, including one hour, two hours and three and a half hours. Veligers in control groups were not exposed to UV; there were separate control groups for each of the three experiments. At the start of each experiment, each of the 16 petri dishes was re-checked to ensure each veliger was alive. Veligers were deemed alive if the veliger was moving; all veligers were active at the beginning of each experiment. Three experiments were run; one at exposure durations of 1, 2, and 3.5 hours. Within each experiment, groups of veligers were exposed to UV at radiation levels 0.5 mW/cm², 0.3 mW/cm² and 0.1 mW/cm².
After a two week delay, veligers were examined and observed for movement, internal tissue condition (present/decomposed) and shell position (gaping/closed) to determine mortality. The delay ensured that a short-term stunned condition would not be mistaken for mortality. If veligers had no internal movement for at least two minutes, they were deemed dead (Figure 22).

Figure 22: After exposing veligers to UV radiation, veliger condition was checked to determine mortality. Most veligers under control conditions remained alive and active through the experiment (top picture) and veligers that experienced mortality (bottom picture) were counted.
7.6 Statistics & Data Analysis:

The dose-response function was calculated using mortality (%) on the y axis and total dose (UV intensity times time) on the x axis. Dose-response functions were fitted to the data using OriginPro9 (OriginLab® Data Analysis and Graphing Software); goodness of fit was determined using the adjusted $r^2$ value. The adjusted $r^2$ accounts for additional terms that do not improve the model more than they would by chance. The calculated dose-response functions allowed estimation of LD$_{50}$ (the lethal dose required to kill 50% of the animals). All other statistical analysis was performed using JMP 5.0.1 (SAS Institute Inc.) software. Significant difference was determined through the Wilcoxon Test. Arithmetic mean and standard error was calculated using Excel.
CHAPTER 8—RESULTS

8.1 Long-Term (Chronic) Exposure Experiments – Adult & Juvenile Mussels:

Mussels exposed to UV were observed closing their shells within the first half hour of exposure and remained closed or nearly closed for the remainder of the experiment. Each mussel exposed to UV was not observed extending its siphon or its foot to move within the container. Mussels under control conditions were observed actively siphoning and moved throughout the experiment (Figure 23). Mussels under control conditions were observed attaching to the container.

Figure 23: In experiments testing UV on *Dreissena bugensis*, a control group was not exposed to UV. Mussels in the control group were observed actively siphoning throughout the experiment.
Adult and juvenile mussel survivorship decreased as total dose increased (Figure 24 and 25). No juvenile mussel survived past 72 hours of UV-B exposure and no adult mussel survived past 20 days of UV-B exposure. Over 90% of all juvenile mussels died after 61 hours and over 90% of all adult mussels died after one week of exposure to UV-B. All mussels survived under control conditions. For both juveniles and adults, survivorship decreased with increased UV intensity.

Figure 24: Time to 100% mortality for juvenile Lake Mead *Dreissena bugensis* is displayed as the mean with standard error for three different UV-B intensity levels, 0.1, 0.3, 0.5 mW/cm² plus control.
Figure 25: Time to 100% mortality for adult Lake Mead *Dreissena bugensis* is displayed as mean with standard error for three different UV-B intensity levels, 0.1, 0.3, 0.5 mW/cm² plus control.

The average time adult mussels took to reach 100% mortality when exposed to 0.1, 0.3 and 0.5 mW/cm² was 10, 6.6 and 4.4 days, respectively. No significant difference was seen in the time it took to reach 100% mortality among intensity levels; the chi² value was 6.1 and p=0.47. The dose-response function fitted to adult mussel mortality as a response to dose, total UV-B, (Table 2 & Figure 26) fit to the data with an adjusted r² value of 0.74 (p=2.2) and chi² of 330. The LD₅₀ calculated from this function is 44,255 mJ/cm².
Figure 26: Lake Mead adult *Dreissena bugensis* mortality (%) as a response to total dose UV-B exposure.

On average, it took a significantly longer time to reach 100% mortality when juveniles were exposed to 0.1 mW/cm² compared to 0.3 and 0.5 mW/cm² intensity levels ($\chi^2=6.2$ and $p=0.044$). The dose-response relationship for juvenile mussels fit with an adjusted $r^2$ value of 0.44 and chi$^2$ of 690 (Figure 27 & Table 2). The LD$_{50}$ calculated from this function was 11,000 mJ/cm$^2$. 
8.2 Short-Term (Acute) Exposure Experiments – Veligers:

Dose response relationships for veligers show a trend of increasing dose with increasing mortality. Veligers under control conditions (no UV exposure) survived at a rate of 97%. The dose-response function fitted to veliger mortality (response) and dose (total UV-B) did not fit as well as the other two functions, with an adjusted $r^2$ value of 0.30 and chi$^2$ value of 40,000 (Figure 28 & Table 2). The LD$_{50}$ value calculated using this function was 860 mJ/cm$^2$. Table 2 summarizes statistics and results to compare veligers, juveniles and adults. As Dreissena grow and proceed through their life history, the
amount of UV needed to kill them increases, which can be seen in the LD\textsubscript{50} values, which increase as the \textit{Dreissena bugensis} proceed from veliger to juvenile to adult.

![Figure 28: Lake Mead \textit{Dreissena bugensis} veliger mortality (%) as a response to total dose UV-B exposure; data from all three experiments is presented. The mortality percent was calculated from each petri dish.](image)

When veligers were exposed to the same intensity level for different time durations, no significant difference was found in average mortality. Specifically, when exposed to 0.1 mW/cm\textsuperscript{2} for one hour, two hour or three and a half hour durations, no significant difference was observed with a \textit{chi}\textsuperscript{2} of 1.2 and p=0.55. After being exposed for one hour with 0.1 mW/cm\textsuperscript{2}, average mortality was 25\% (standard error 16.0). After
being exposed for two hours, average mortality percentages were 8.3 (standard error 8.3) and after three and a half hours of exposure at 0.1 mW/cm$^2$ the average percent mortality was 33 (standard error 19).

After three and a half hours of UV-B there was a significant difference in mortality percentages between veligers exposed to 0.1 and 0.3 mW/cm$^2$, but no significant difference in mortality percentages between 0.3 and 0.5 mW/cm$^2$; the chi$^2$ value was 8.6 and $p=0.014$. Similarly, after two hours of UV-B exposure, mortality was significantly higher between veligers exposed to 0.1 mW/cm$^2$ and 0.3 mW/cm$^2$. The chi$^2$ value was 9.3 and $p=0.0096$ indicating a significant difference between mortality rates in response to 0.1 mW/cm$^2$ and 0.3 mW/cm$^2$. After two hours of exposure, mortality percentages were 8.3% (standard error of 8.3), 83% (standard error of 9.6) and 100% (standard error 0).
Table 2: Dose-response functions, statistics and calculated LD$_{50}$ for adult, juvenile and veliger quagga mussels.

<table>
<thead>
<tr>
<th>Life-Stage</th>
<th>Fig.</th>
<th>Adjusted $r^2$</th>
<th>Reduced Chi$^2$</th>
<th>Dose-Response Function</th>
<th>LD$_{50}$ (mJ/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>2</td>
<td>0.74</td>
<td>328</td>
<td>$Response% = \frac{92.75855 - 95.63318}{1 + \left(\frac{Dose}{40194.58656}\right)^{2.20676}}$</td>
<td>44,255</td>
</tr>
<tr>
<td>Juvenile</td>
<td>3</td>
<td>0.44</td>
<td>692</td>
<td>$Response% = -147.55946 + 22.22514\ln(Dose - 3285.39973)$</td>
<td>10,537</td>
</tr>
<tr>
<td>Veliger</td>
<td>4</td>
<td>0.30</td>
<td>40,000</td>
<td>$Response% = \frac{96.47921 - 51.89827}{1 + \left(\frac{Dose}{1002.1979}\right)^{9.21759}}$</td>
<td>857</td>
</tr>
</tbody>
</table>
CHAPTER 9—DISCUSSION

9.1 Chronic Exposure:

Behavioral differences were observed in mussels exposed to UV compared to mussels under control conditions. Mussels exposed to UV generally remained closed for the duration of the experiment, and kept their feet retracted. Conversely, mussels in the control groups moved within the container and actively siphoned. These observations were similar to those observations made by Chalker-Scott et al. (1993). In a review by Williamson (1995), it was noted that organisms that cannot detect damaging UV-B wavelengths were more susceptible UV damage. In this study, adult and juvenile *Dreissena bugensis* responded to UV-B radiation, but it is not clear if they can detect those wavelengths with photoreceptors or if their behavior is a response to UV damage or photorepair processes. As the mussels that were exposed to UV closed their shells and reduced siphoning, partial starvation may have resulted. Starvation stress could have contributed to mortality but starvation cannot fully explain mortality rates seen in this study because *Dreissena* are highly starvation resistant, McMahon (1996) noted that *Dreissena polymorpha* experienced only 50% mortality after 118 days and 100% mortality after 352 days.

The larger the mussels were, the more resistant they were to UV-B, but their resistance may be a function of size or life stage. The apparent size dependency is consistent with results for other invertebrates. This has been found on other invertebrates as well; e.g., McNamara and Hill (1999) found that larger snails were more resistant to UV-B than smaller snails. Smaller animals may be more easily affected by UV because although the total number of damaged cells is likely the same, the proportion of damaged
cells to the total number of cells in the animal would be greater in smaller animals, which have a smaller number of total cells. Smaller animals also have a larger surface area to volume ratio, which means that the cells most likely to be affected by UV, the surface cells, account for a larger portion of the entire animal.

Dose-response functions fit to well to adult data plotted with percent mortality as the response to dose, UV-B exposure. For adult mussels the logistic function fitted to the data shows a trend of increasing effectiveness with increasing dose, and a leveling off around 1x10^5 mJ/cm^2. “S”-shaped, logistic or sigmoid-shaped functions have been similarly found to fit for percent mortality of aquatic organisms in response to UV-B dose. This makes sense because at low doses, UV is not effective. At moderate doses, mortality increases, and at higher doses, mortality cannot increase at the same rate because most animals have already perished. The slope of the dose-response curve flattens at higher doses as some mussels appear to be UV-B resistant, surviving much longer than the majority of the mussels. Wubben (2000) found a similar “s”-shaped dose–response function for shrimp larvae’s response to UV-B exposure. McNamara and Hill (1999) also found a logistic function fit well to the mortality response of aquatic organisms to UV dose. The dose-response function for adult mussels fit better than that for juveniles. For adults, the function had an adjusted r^2 value of 0.74, compared to an adjusted r^2 value of 0.44 for juveniles. Individual variability in UV resistance in juveniles is higher than the variability in adults. Juvenile mussels’ resistance is more varied than adult mussels’ resistance. Higher variability in juvenile resistance could be because by the time the mussels have reached adulthood, only the more fit mussels have survived environmental conditions to reach that life stage.
LD$_{50}$, increases as the *Dreissena bugensis* progress in their life stage. Estimated LD$_{50}$ values for adult and juvenile mussels were about one to two orders of magnitude greater than several other aquatic species previously studied by McNamara and Hill (1999), Wubben (2000) and Lacuna and Uye (2001) including *Copepod* and *Physella*. The differences in LD$_{50}$ estimated in this study are reasonably similar to other aquatic species, but variability could be due to how other studies’ experimental design, UV-B wavelength, or the presence of photorepair in various species. *Dreissena bugensis* are also likely to have a higher tolerance for UV-B due to their larger size and protective shells when compared to species such as *Diphetor, Daphnia* and *Copepod*. When compared to *Physella* (McNamara and Hill, 1999), the estimated LD$_{50}$ for *Dreissena bugensis* was about one order of magnitude higher, which may reflect *Dreissena bugensis*’s intrinsic ability as invasive species to better resist environmental stress to outcompete other aquatic organisms.

Among juvenile and adult *Dreissena bugensis*, a small portion of mussels in each group survived much longer than the majority, indicating that some *Dreissena bugensis* are relatively UV-B resistant. This could be due to shell characteristics or thickness or could be the result of the varying presence of higher levels of mycosporine-like amino acids, (MAAs) similar to those discovered in the mussel, *mytilus galloprovincialis* (Chioccaro et al., 1979; Chioccaro et al., 1985). Because the experimental mussels had very little exposure to additional light outside the UV spectrum it is unlikely that photorepair was more active in UV-resistant mussels than mussels that died more quickly.

Damkaer and Dey (1983) found that shrimp larvae could survive UV doses under a certain level by using photorepair to recover. Additional studies should be done to
determine if *Dreissena* can use MAAs or photorepair to survive UV. Garcia-pichel (1983) found that MAAs exhibited wavelength dependence and Damkaer and Dey (1983) cited that photoreactivating enzymes were most effective in wavelengths between 315 and 500 nm. Additional research might provide information on the wavelengths that are most effective for photorepair so that only damaging wavelengths are engineered into UV control systems. Photoreactivation could be a consideration in certain engineering configurations; in areas receiving more natural light, the UV intensity/dose may need to be increased if the mussels utilize other wavelengths to recover from UV-B damage.

The basic dose-response functions modeled in this study can be used as a baseline for additional investigation. Further research should be done to narrow the most effective wavelength for this species and investigate additional synergistic effects of combining UV-B with other stressors, such as copper and reduced calcium levels, to increase *Dreissena bugensis* mortality. Combined oxidant treatments using synergistic effects in attempt to reduce necessary concentrations have recently been studied (Costa et al., 2012; Costa et al., 2011) and this concept can be applied to using UV-B to control *Dreissena bugensis*. Palenik et al. (1990) cited studies showing that UV-B can alter metals making them more toxic and/or more available to organisms, noting copper, specifically. Copper is known to be toxic to *Dreissena* (Faria et al., 2010; Bouskill et al., 2006; Ivankovic, et al., 2009) by causing oxidative stress; it can also cause lipid peroxidation (Bouskill et al., 2006). Hessen and Rukke (2000) found that *Daphnia* became significantly more susceptible to UV damage in conditions of reduced calcium. There is evidence that *Dreissena* are limited by environmental calcium concentrations (Whittier et al. 2008) because of their shells are primarily made up of calcium. Therefore, further studies
should be done examining whether UV is more damaging to *Dreissena bugensis* in low calcium waters.

9.2 Acute Exposure:

Veliger mortality increased with increased UV-B exposure while 97% of the control veligers survived the duration of the experiment. Dose-response relationships for adults and juveniles showed a trend of increasing dose with increasing mortality; a similar pattern was seen for veligers. Veliger survivorship among the control group was high (97%). The survival rates of control veligers here is similar to control survivorship in similar studies, for example Wubben (2000) had >80% survivorship in control animals, and Hori et al. (1990) had 95% to 86% survivorship rates among control animals. The dose-response function fitted to veliger data did not fit as well as the juvenile and adult data, with an adjusted $r^2$ value of 0.30 and chi$^2$ value of 40,000 (Figure 4 & Table 2). In this study, veligers could have used photorepair to survive UV because the veligers were kept in an incubator with partial light; if some internal recovery was occurring, this would make the data more irregular, and the dose-response curve would not fit as well. In a hypothetical water intake system UV would likely be engineered in a way in which veligers were exposed to UV, and then flowed through the system back into daylight. Additional research should be done to determine if *Dreissena bugensis* veligers can use photorepair to recover from UV-B exposure. Estimated LD$_{50}$ for veligers was about two orders of magnitude lower than that of juveniles and adults, which appear reasonable for their smaller size and more transparent shells. The LD$_{50}$ for veligers was about one order of magnitude lower than many other aquatic species (Table 2). Although Wright et al. (1997) did not calculate the LD$_{50}$, they found that a dose of 1,156.5 mJ/cm$^2$ at a
wavelength of 297 nm led to *Dreissena polymorpha* veliger mortality rates of about 69%. The LD$_{50}$ for *Dreissena bugensis* veligers estimated in this study is 857 mJ/cm$^2$ at a similar wavelength of 306 nm. This experimental design may have led to higher survivorship because veligers were less crowded in petri dishes. While our results agreed generally with those of Wright et al. (1997), our results did not agree with Chalker-Scott et al. (1994) who found much lower levels of UV needed for high mortality rates. The difference could be due to the experimental set-up or Lake Mead *Dreissena bugensis* veligers may be more resistant to UV than the *Dreissena polymorpha* veligers tested by Chalker-Scott (1994).

9.3 Comparisons between Chronic and Acute Studies:

Of the veligers and juvenile mussels that were exposed for two or more hours, there was a significant increase in mortality between those animals exposed to 0.1 and 0.3 mW/cm$^2$, which means that a threshold of increased intensity lethality may exist between 0.1 and 0.3 mW/cm$^2$ for juveniles and veligers. Dose-response functions differed between adult/juveniles and veligers. A much lower UV-B dose, about one order of magnitude, is needed to kill veligers compared to juveniles and adults, which means that UV may have higher potential as an engineering solution to treating veligers than adults. The nature of UV as a physical wave that does not dilute in water may still be effective in certain engineered configurations, in which the UV source can be concentrated on a surface that experiences high water flow rates. Damkaer and Dey (1983) found evidence that “repair mechanisms could keep pace with the damaging effects of UV-B” when a dose was administered over a long enough duration of time. Higher irradiation levels rather than a longer exposure duration should be more effective in engineering applications because
lower UV-B intensity levels (0.1 mW/cm²) were significantly less effective than higher levels (0.3 mW/cm²). Higher irradiation levels would limit possible internal photorepair recovery processes.

This study was able to quantify baseline UV-B levels for high *Dreissena bugensis* mortality rates at three life stages. This study was also able to provide dose-response functions to model the pattern that *Dreissena bugensis* respond to UV-B dose in terms of mortality. This information can be used as a starting point for additional research on how UV-B can affectively be used as a control measure to treat and manage *Dreissena bugensis* invasions.
CHAPTER 10—SUMMARY

Lake Mead is composed of multiple basins that have been recently invaded by *Dreissena bugensis*. Little is known about growth rates of *Dreissena bugensis* relative to the water quality characteristics of individual Lake Mead basins. Natural and anthropogenic inputs differ between basins, which creates varying environments for *Dreissena bugensis*. This study provides evidence that *Dreissena bugensis* growth rates vary by basin and are likely affected by the water quality characteristics that also vary by basin. Although nutrient levels were generally higher in Boulder Basin, chlorophyll a and total organic carbon, food sources for *Dreissena bugensis*, were significantly higher for Overton Arm. *Dreissena bugensis* growth rates were higher in Overton Arm compared to Boulder Basin, which may be a result of more food. *Dreissena bugensis* growth rates from each basin were also compared with maximum potential growth rates of *Dreissena bugensis* by providing a separate group with supplementary algae. From this comparison, it appears that *Dreissena bugensis* are growing near their maximum potential growth rate in Overton Arm, but not Boulder Basin. If additional food sources were added to Boulder Basin higher *Dreissena bugensis* growth rates may result. Higher growth rates in Overton Arm compared to Boulder Basin may also be a result of limiting factors in Boulder Basin, which has been more extensively affected by anthropogenic pollutants.

As *Dreissena bugensis* spread through water bodies such as Lake Mead, managing their spread becomes increasingly important, particularly with respect to infrastructure. There are significant drawbacks to the known chemical treatments, suggesting a need for additional strategies. To begin to develop and engineer UV to treat *Dreissena bugensis*, a first step is to quantify the amount required for high mortality rates.
In this study, I was able to model and quantify the amount of UV-B required for high
mortality rates for *Dreissena bugensis* at three life stages including veliger, juvenile and
adult. Dose response functions were fitted to experimental data and median lethal doses
were estimated from these functions, which can be used as a baseline for further
engineering research.
APPENDIX 1 - Collection Permit 1

NEVADA DEPARTMENT OF WILDLIFE

SPECIAL LICENSE/PERMIT

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

| Entity / License/Permittee Name | Thaw Melissa N – Desert Research Institute |
| Mailing Address | 755 E Flamingo Rd |
| City | Las Vegas |
| Street Address | Same |
| City | Same |

| Tax ID/SSN | Date of Birth | Sportsman's ID |
| License Class | 22.02 | Agent No. | 1950 |
| License/Permit Valid From | September 4, 2012 through December 31, 2014 |

S 36161

--- Special Conditions ---

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

- Authorizations and Conditions Attached -

- Period of Collection Activities: See Condition #4 -

- Activity Report(s) Due: 01/30/2014; 01/30/2015 -
PERMITTEE:

Melissa Thaw
Desert Research Institute
755 E Flamingo Rd
Las Vegas NV 89110

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

SCIENTIFIC COLLECTION PERMIT NO. S36161

In compliance with the conditions listed below and pursuant to provisions of NRS 503.597 & 503.850, the permittee, each permit year during the designated sampling period, is authorized to:

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

Prior to any sampling or collection activity at Lake Mead, Permittee must notify NDOW Southern Region biologist Jon Sobert @ (702) 486-5127 ext 3500 or jsobert@ndow.org.

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

CONDITIONS:

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

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

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

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

3. Number Authorized: As indicated above.


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

The record must be submitted to the Nevada Department of Wildlife License Office – Scientific Collection Report, 4500 Kietzke Ln D-135, Reno, NV 89502, by 1/30/14 for 2013 "take" activities and 1/20/15 for 2014 “take” activities. Digital reports in Excel spreadsheet *(preferred)* or Quattro Pro are accepted (please follow column sequence as outlined in the Department report form, 22.85-5.)

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

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

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

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

   Julie Meadows
   Program Officer I
### APPENDIX 2 - SNWA Water Quality Data Sampling Details

<table>
<thead>
<tr>
<th>Site/constituent sampled</th>
<th>Measurement techniques/Sample Method</th>
<th>Depth of measurement (meters)</th>
<th>Measurement Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB_7 Orthophosphate</td>
<td>Not available/Unknown</td>
<td>Variable: 0, 22, 35, 19, 38</td>
<td>1-3 times a month</td>
</tr>
<tr>
<td>BB_7 Nitrate</td>
<td>Not available/Unknown</td>
<td>Variable: 0, 22, 19, 38, 16</td>
<td>1-3 times a month</td>
</tr>
<tr>
<td>BB_3 Total Organic Carbon</td>
<td>Grab</td>
<td>Variable: 5, 30, 60</td>
<td>Once a month</td>
</tr>
<tr>
<td>BB_7 Chlorophyll a</td>
<td>Not Available/Unknown</td>
<td>0 m, 2.55 m</td>
<td>1-2 times a month</td>
</tr>
<tr>
<td>VR_13 Orthophosphate</td>
<td>Not Available/Unknown</td>
<td>Variable: 0, 5, 20, 60 m</td>
<td>Once a month</td>
</tr>
<tr>
<td>VR_13 Nitrate</td>
<td>Not Available/Unknown</td>
<td>Variable: 0, 5, 20, 60</td>
<td>Once a month</td>
</tr>
<tr>
<td>VR_13 Total Organic Carbon</td>
<td>Grab</td>
<td>Variable: 0, 1, 5, 20, 30, 40, 50, 60, 62</td>
<td>Once a month</td>
</tr>
<tr>
<td>VR_13 Chlorophyll a</td>
<td>Not Available/Unknown</td>
<td>0, 5</td>
<td>Once a month</td>
</tr>
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</table>
## APPENDIX 3 – Statistical Details – Lake Mead Basin Growth Experiments

<table>
<thead>
<tr>
<th>Data/ Experiment</th>
<th>Normal (Y/N)</th>
<th>Test Run</th>
<th>Results/Notes/P Value</th>
<th>N/n</th>
<th>Mean of Entire Population/Sample</th>
<th>Standard Deviation of Entire Population/Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA v. BB Growth – Wet Weight incl. shell + tissue</td>
<td>Yes (p=0.840)</td>
<td>ANOVA Student t test</td>
<td>P=0.0048 BB &amp; EB are sig different EB &amp; MG – not significantly different BB &amp; MG are sig different</td>
<td>18</td>
<td>0.0748 somatic growth rate</td>
<td>0.0341</td>
</tr>
<tr>
<td>LMM &amp; CB</td>
<td>No (p=0.057)</td>
<td>Wilcoxon-Kruskal-Wallis</td>
<td>LMM &amp; CB – not different MG different from LMM &amp; CB (p=0.0233)</td>
<td>18</td>
<td>0.0395 somatic growth rate</td>
<td>0.0316</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>No (p&lt;0.0001)</td>
<td>Wilcoxon/Kruskal-Wallis</td>
<td>P=0.0317</td>
<td>31</td>
<td>0.562 mg/m³</td>
<td>0.393</td>
</tr>
<tr>
<td>Nitrate</td>
<td>No (p&lt;0.0001)</td>
<td>Wilcoxon/Kruskal-Wallis</td>
<td>P&lt;0.001</td>
<td>76</td>
<td>0.520 mg/L</td>
<td>0.182</td>
</tr>
<tr>
<td>Orthophosphate</td>
<td>No (p&lt;0.0001)</td>
<td>Wilcoxon/Kruskal-Wallis</td>
<td>P&lt;0.001</td>
<td>334</td>
<td>1.270 µg/L</td>
<td>0.844</td>
</tr>
<tr>
<td>Total Organic Carbon</td>
<td>No (p&lt;0.001)</td>
<td>Wilcoxon/Kruskal-Wallis</td>
<td>P&lt;0.0001</td>
<td>204</td>
<td>2.70 mg/L</td>
<td>0.342</td>
</tr>
</tbody>
</table>

[1] OA=Overton Arm  
BB=Boulder Basin  
LMM=Lake Mead Marina  
CB=Callville Bay  
MG = Maximum Potential Growth  
## APPENDIX 4 Growth Experiments and Water Characterization Descriptive Statistics

<table>
<thead>
<tr>
<th>Data</th>
<th>Time of Year</th>
<th>N/n</th>
<th>Mean Value</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB Dreissena Somatic Growth Rate&lt;sup&gt;2&lt;/sup&gt;</td>
<td>April 2012</td>
<td>6</td>
<td>0.0448</td>
<td>0.0109</td>
</tr>
<tr>
<td>OA Dreissena Somatic Growth Rate&lt;sup&gt;2&lt;/sup&gt;</td>
<td>April 2012</td>
<td>6</td>
<td>0.07698</td>
<td>0.00719</td>
</tr>
<tr>
<td>MG Dreissena Somatic Growth Rate&lt;sup&gt;2&lt;/sup&gt;</td>
<td>April 2012</td>
<td>6</td>
<td>0.103</td>
<td>0.0124</td>
</tr>
<tr>
<td>LMM Dreissena Somatic Growth Rate&lt;sup&gt;3&lt;/sup&gt;</td>
<td>November 2011</td>
<td>6</td>
<td>0.0241</td>
<td>0.00818</td>
</tr>
<tr>
<td>CB Dreissena Somatic Growth Rate&lt;sup&gt;3&lt;/sup&gt;</td>
<td>November 2011</td>
<td>6</td>
<td>0.0314</td>
<td>0.01498</td>
</tr>
<tr>
<td>MG Dreissena Somatic Growth Rate&lt;sup&gt;3&lt;/sup&gt;</td>
<td>November 2011</td>
<td>6</td>
<td>0.0630</td>
<td>0.0102</td>
</tr>
<tr>
<td>BB Chlorophyll a&lt;sup&gt;4&lt;/sup&gt;</td>
<td>March, April, May</td>
<td>17</td>
<td>0.5 mg/m&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.0959</td>
</tr>
<tr>
<td>EB Chlorophyll a&lt;sup&gt;4&lt;/sup&gt;</td>
<td>March, April, May</td>
<td>14</td>
<td>0.642 mg/m&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.0999</td>
</tr>
<tr>
<td>BB Total Organic Carbon&lt;sup&gt;4&lt;/sup&gt;</td>
<td>March, April, May</td>
<td>157</td>
<td>2.333 mg/L</td>
<td>0.0211</td>
</tr>
<tr>
<td>OA TOC&lt;sup&gt;4&lt;/sup&gt;</td>
<td>March, April, May</td>
<td>43</td>
<td>2.804 mg/L</td>
<td>0.0246</td>
</tr>
<tr>
<td>BB Nitrate&lt;sup&gt;4&lt;/sup&gt;</td>
<td>March, April, May</td>
<td>44</td>
<td>0.662 mg/L</td>
<td>0.00793</td>
</tr>
<tr>
<td>OA Nitrate&lt;sup&gt;4&lt;/sup&gt;</td>
<td>March, April, May</td>
<td>30</td>
<td>0.314 mg/L</td>
<td>0.0118</td>
</tr>
<tr>
<td>BB Orthophosphate&lt;sup&gt;4&lt;/sup&gt;</td>
<td>March, April, May</td>
<td>41</td>
<td>1.83 µg/L</td>
<td>0.0589</td>
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<tr>
<td>OA Orthophosphate&lt;sup&gt;4&lt;/sup&gt;</td>
<td>March, April, May</td>
<td>291</td>
<td>1.19 µg/L</td>
<td>0.0504</td>
</tr>
</tbody>
</table>

[1] OA=Overton Arm  
BB=Boulder Basin  
LMM=Lake Mead Marina  
CB=Callville Bay  
MG = Maximum Potential Growth  

[2] Measured wet weight (shell+tissue)  

[3] Measured dry weight (tissue only)
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(Dreissena bugensis) into Lake Mead, Nevada-Arizona. Lake and Reservoir Management, 26(4) doi:10.1080/07438141.2010.504071


VITA

Graduate College
University of Nevada, Las Vegas

Melissa Nicole Thaw

Local Address:
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Degrees:
Bachelor of Arts, East Asian studies, 2004
Lewis & Clark College

Thesis Title: Understanding basin specific life history characteristics of lake mead quagga mussels (Dreissena bugensis) and a potential treatment using UV radiation in laboratory studies

Thesis Examination Committee:
Dr. Kumud Acharya, Ph. D.
Dr. Michael J. Nicholl, Ph. D.
Dr. Carl Reiber, Ph. D.
Dr. Craig Palmer, Ph. D.