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Sean Robin Comeau
University of Nevada, Las Vegas

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COLONIZATION AND DECONTAMINATION OF QUAGGA MUSSELS IN THE WESTERN UNITED STATES: MONITORING VELIGERS IN LAKE MEAD AND FIELD TESTING THE EFFECTS OF HOT-WATER SPRAY AS A MEANS OF WATERCRAFT DECONTAMINATION

by

Sean Robin Comeau

Bachelor of Science
University of Nevada, Reno
2009

A thesis submitted in partial fulfillment of
the requirements for the

Master of Public Health
Department of Environmental and Occupational Health
School of Community Health Sciences
Division of Health Sciences

Graduate College
University of Nevada, Las Vegas
May 2011

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THE GRADUATE COLLEGE

We recommend the thesis prepared under our supervision by

Sean Robin Comeau

entitled

Colonization and Decontamination of Quagga Mussels in the Western United States: Monitoring Veligers in Lake Mead and Field Testing in the Effects of Hot-Water Spray as a Means of Watercraft Decontamination

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May 2011

ABSTRACT

Colonization and Decontamination of Quagga Mussels in the Western United States: Monitoring Veligers in Lake Mead and Field Testing the Effects of Hot-Water Spray as a Means of Watercraft Decontamination

by

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The introduction and establishment of aquatic invasive species (AIS) is one of the top causes of global biodiversity loss and ecological change, and is also financially costly for taxpayers and agencies managing protected areas. This is especially true regarding the nationwide spread of dreissenid mussels to various bodies of freshwater. The discovery of invasive quagga mussels (*Dreissena rostriformis bugensis*) in Lake Mead on January 6, 2007 has changed the popular recreation area into a potential vector of the destructive AIS. This location could allow for an increased spread of these dressenid species to uninfested bodies of water in the western United States due to overland transport of contaminated watercraft. In order to prevent further infestations, new information must be used to generate and revise uniform minimum protocols and standards for watercraft decontamination programs. Protocols regarding safe and inexpensive procedures, such as hot-water sprays, which result in the 100% mortality of quagga mussels, need to be created. Current protocol regarding zebra mussels may not be applicable to quagga mussels due to an increased susceptibility. Emersed adult quagga mussels were exposed to hot-water sprays at 20, 40, 50, 54, 60, 70, and 80°C for 1, 2, 5, 10, 20, 40, 80, and 160

s. Sprays at ≥60°C for 5 s were shown to be 100% lethal. Sprays of 54°C for 10 s, 50°C for 20 s, and 40°C for 40 s also resulted in 100% mortality. A spray temperature of 60°C for 5 s is recommended for mitigating fouling by quagga mussels. Inaccessible areas and areas with special heat requirements on watercraft were also evaluated and field tests on actual quagga encrusted watercraft were performed under summer and winter conditions, respectively. This study also determined veliger abundance and colonization rates during different months of the year, which is helpful for anti-fouling management strategies.

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TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGEMENTS v

LIST OF TABLES ix

LIST OF FIGURES x

CHAPTER 1 INTRODUCTION 1
Purpose of the Study 4
Research Questions 6
Hypotheses 6
Significance of the Study 9

CHAPTER 2 REVIEW OF RELATED LITERATURE 10
Aquatic Invasive Species 10
Economic Impact and Spread 10
Dreissenid Mussels 11
Quagga Mussel Biology 13
Life Cycle 13
Morphological Differences between the Quagga Mussel and the Zebra Mussel 14
Containment of Existing Dreissenid Populations 15
Watercraft Decontamination 15
Chemical Decontamination 16
Heat 17
Hot-Water, High-Pressure Washing 17
Freezing 18
Physical 19
Desiccation 19

CHAPTER 3 METHODOLOGY 20
Hot-Water Spray Decontamination of Quagga Mussels 20
Field Tests on Emersed Quagga Mussels 22
Specimen Collection and Holding Conditions 22
Experimental Design and Measurements 22
Data Collection and Management 24
Statistics and Data Analysis 25
Evaluation of Category II Watercraft Areas 26
Evaluation of Category III Watercraft Areas 26
Summer and Winter Validation 27
Veliger Collection and Substrate Colonization 27
Method of Veliger Collection and Analysis 27
Method of Substrate Collection and Analysis 30

CHAPTER 4 FINDINGS OF THE STUDY ... 32
Results from Field Tests on Emersed Quagga Mussels ... 32
Results from the Evaluation of Category II Areas ... 35
Results from the Evaluation of Category III Areas ... 36
Boat Decontamination Validation Results ... 38
Summer Validation ... 38
Winter Validation ... 40
Results of Veliger Collection at Specific Depths in Lake Mead ... 41
Results of Veliger Colonization at Specific Depths in Lake Mead ... 45

CHAPTER 5 DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS ... 46
Discussion of Results ... 46
Susceptibility of Quagga Mussels to Hot-Water Sprays ... 46
Field Validation of Category I and Category II Watercraft Areas ... 50
Veliger Collection and Substrate Colonization at Different Depths ... 54
Discussion of Research Questions ... 58
Study Limitations ... 60
Study Contributions ... 61
Conclusions and Recommendations for Further Study ... 62

APPENDIX 1 PROTOCOLS ... 65

APPENDIX 2 DATA TABLES ... 75

REFERENCES ... 80

VITA ... 84

LIST OF TABLES

Table 1 Accessibility Categories for Various Decontamination Areas 2
Table 2 Amount of Adult Quagga Mussels Tested per Treatment Group 24
Table 3 Sampling Volume Calculated per Tow at Specific Depths 29
Table 4 Quagga Mussel Mortality (%) under Different Treatments at Day 10 32
Table 5 Estimated LT_{50} and LT_{99} Values (in bold) and their 95% Confidence Limit for Hot-Water Spray Treatments on Quagga Mussels at 1 s, 2 s, and 5 s Application Durations 34
Table 6 Shell Length (mm) of Quagga Mussels for the Hot-Water Spray Experiment 35
Table 7 Evaluation of Lethal Temperature Recommendations on the Gimbal Unit during Summer Weather Conditions 36
Table 8 Evaluation of Lethal Temperature Recommendations on the Bait Wells during Winter Weather Conditions 36
Table 9 Evaluation of Lethal Temperature Recommendations on the Bait Wells during Summer Weather Conditions 37
Table 10 Evaluation of Lethal Temperature Recommendations on the Bait Wells during Summer Weather Conditions 37
Table 11 Evaluation of Lethal Temperature Recommendations on the Live Wells during Summer Weather Conditions 37
Table 12 Evaluation of Lethal Temperature Recommendations on the Live Wells during Summer Weather Conditions 38
Table 13 Number of mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category I Areas (summer) 39
Table 14 Number of mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category II Areas (summer) 39
Table 15 Number of mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category I Areas (winter) 40
Table 16 Number of mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category I Areas (winter) 41

LIST OF FIGURES

Figure 1 Examples of Watercraft Areas ... 3
Figure 2 Dreissenid Distributions in North America ... 12
Figure 3 External Morphology of the Zebra Mussel and the Quagga Mussel ... 15
Figure 4 Mortality (%) of Quagga Mussels in Lake Mead after Hot-Water Spray Treatment ... 33
Figure 5 Total Veliger Abundance per L Calculated by Depth ... 42
Figure 6 Average Pediveliger Abundance per L Calculated by Depth ... 43
Figure 7 Average Umbonal Veliger Abundance per L Calculated by Depth ... 43
Figure 8 Average D-Shaped Veliger Abundance per L Calculated by Depth ... 44
Figure 9 Average Trochophore Veliger Abundance per L Calculated by Depth ... 44
Figure 10 Biweekly Veliger Settlement at Specific Depths in Lake Mead from June 30, 2010 to December 28, 2010 ... 45

CHAPTER 1

INTRODUCTION

The introduction and establishment of aquatic invasive species (AIS) in freshwater bodies across the United States has been shown to have an extensive deleterious impact on the economic potential and environmental stability of the affected regions (Leung et al. 2006; Piola 2009). It has been widely accepted that much of the spread of AIS to previously uncontaminated bodies of freshwater can be attributed to the overland movement of contaminated trailered boats (Bossenbroek et al. 2001; Johnson et al. 2001; Leung et al. 2006). Two AIS of particular importance to being unintentionally transferred are the zebra mussel (*Dreissena polymorpha)* and the quagga mussel *(Dreissena rostriformis bugensis).* The small size and resilience of these dreissenid species enable them to avoid detection during boat inspection and to remain viable for several days during overland transport from a contaminated body of water (Ricciardi 1998). The discovery of quagga mussels in the western United States at Lake Mead, NV, and zebra mussels in San Justo Reservoir, CA, has caused many government agencies to initiate watercraft interception programs to prevent further infestations by these invasive mussels (Zook and Phillips 2009). Many of these agencies have protocols that commonly decontaminate watercraft with a pressurized hot-water spray exceeding 140°F/60°C. This temperature is based on acute (short-term) upper-thermal limit data generated for continuously immersed mussels (Morse 2009). The first data set on the use of hot-water spray for mitigation of emersed zebra mussel fouling, which is closer to the field situation where sprays are applied to watercraft, was generated by Morse (2009). Morse found that the survivorship of mussels was affected by two major factors: spray water temperature

and exposure duration. Water sprayed at ≥140°F/60°C for 10 s or 176°F/80°C at ≥5 s was 100% lethal to zebra mussels, which indicates that current decontamination recommendation of spray temperature of ≥140°F/60°C may not result in 100% mortality of the mussels if the exposure duration is <10 s (Morse 2009).

The data from Morse (2009) can be potentially applied to watercraft areas where the spray directly contacts the fouled areas (Category I areas in Table 1). Concurrently, there are also areas on watercraft that hot-water sprays cannot directly reach. These decontamination areas can be divided into three categories: (1) areas easy to access; (2) areas difficult to access; and (3) special areas (Table 1, Figure 1). These three categories of areas should be treated differently to achieve 100% quagga mussel mortality for legitimate watercraft and equipment decontamination.

Table 1. Accessibility Categories for Various Decontamination Areas.

Category	Characteristics	Areas
I	Easy access surface areas	hull, transducer, through hull fittings, trim tabs, zincs, centerboard box and keel (sailboats), foot-wells, lower unit, cavitation plate, cooling system intakes (external), prop, prop shaft, bolt heads, engine housing, jet intake, paddles and oars, storage areas, splash wells under floorboards, bilge areas, drain plug, anchor, anchor and mooring lines, PFD's, swim platform, inflatables, down-riggers and planing boards, ice chests, fishing gear, bait buckets, stringers, trailer rollers and bunks, light brackets, cross-members, license plate bracket, fenders, spring hangers
II	Hard access areas	gimbal areas, engine, generator, and AC cooling systems (internal)
III	Special areas that require water temperature ≤ 130°F for decontamination	ballast tanks/bladders, washdown systems, bait and live wells, internal water systems

In order to develop proper watercraft decontamination standards, the areas not capable of receiving direct spray or not capable of withstanding the recommended 140°F/60°C temperature must be evaluated. In addition to this, there are several important aspects that need to be addressed regarding species-specific application. This is a key component that concerns existing agency protocol because some freshwater bodies of water may be infested with only quagga mussels, only zebra mussels, or a combination of both. In the western United States this is of particular importance because quagga mussels are currently the most widespread dreissenid species with only one freshwater body infested by zebra mussels (Benson 2011). Many previous studies have shown that there are some differences between these two dreissenid species (Baldwin 2002; Mills 1995; Pathy 1993; Peyer et al. 2009; Ricciardi 1995). It is important to evaluate the susceptibility of quagga mussels to hot-water sprays to determine if they are more or less susceptible than zebra mussels. This information is helpful in making standards for watercraft interception programs (Zook and Phillips 2009).

Figure 1. Examples of Watercraft Areas; (a) Category I: Hull; (b) Category II: Gimbal area; (c) Category III: Ballast system.

In bodies of waters in the western United States where the quagga mussel has already been established, it important to monitor the dreissenid species to provide information

about population dynamics and ecosystem impacts for the purpose of enhancing resource management. Much of the data regarding the life history of the dreissenid mussels in North America comes from their early colonization of the Great Lakes region. There are, however, several important differences between freshwater bodies in the west and the Great Lakes region such as water quality, salinity, temperature, etc. Most importantly, the average water temperature is much higher in the lower Colorado River than in the Great Lakes region (Meuting 2009). Information regarding the life cycle and behavior of quagga mussels in the arid southwest needs to be evaluated in order to determine the specific periods during the year when and where the highest settlement rates occur and highest concentrations of competent pediveligers are present in order to manage anti-fouling practices most efficiently.

Purpose of the Study

The primary purpose of the first study was to determine the minimum thresholds (temperature and duration) of pressurized hot-water sprays that result in 100% quagga mussel mortality as a potential method of watercraft decontamination. This study was designed to provide valid field data on the most efficient use of hot-water sprays to decontaminate watercraft and equipment infested by quagga mussels by systematically testing combinations of temperatures and application duration. For this study, the field experiments were used: (1) to establish the relationships between quagga mussel mortality, hot-water spray temperatures, and exposure times for watercraft areas that can be directly exposed to high-temperature spray water; (2) to determine the minimum amount of time required to reach and sustain the lethal temperature in watercraft areas

(i.e. gimbal areas) that can only be indirectly exposed to hot-water sprays; (3) to ascertain the time necessary to reach and sustain the lethal temperature in watercraft areas (i.e. ballast tanks) with special temperature requirements; and (4) to validate the experimental data by decontaminating actual watercraft and equipment infested with quagga mussels at Lake Mead National Recreation Area with various combinations of spray water temperatures and exposure durations to achieve 100% quagga mortality.

The primary purpose of the second study was to determine the weekly quagga mussel veliger abundance and biweekly settlement rates at several different depths in Lake Mead, NV, from June 30, 2010 until December 28, 2010. This is a portion of quagga mussel monitoring protocol currently in place by the National Park Service which will be continued for a full year. The information can be used to determine the precise temporal presence of the competent pediveligers to settle on substrates at specific depths in a freshwater body in the southwest United States. Knowledge of this particular data is critical for implementing anti-fouling measures for this particular region in a timely and efficient way.

Protocols for boat decontamination using hot-water spray have already been established for dreissenid mussels, mainly the zebra mussel, but before this study it had not been evaluated if the quagga mussel is more or less susceptible to this established threshold than the zebra mussel. This unknown association and the aforementioned study objectives were the basis for both of these studies research questions and subsequent hypotheses.

Research Questions

- What are the temperatures and exposure times needed to attain 100% mortality of adult quagga mussels following exposure to a hot-water spray?
- Is the quagga mussel more or less susceptible than the zebra mussel to hot-water spray?
- For those watercraft areas that are inaccessible to spray treatment (i.e. gimbal areas); how long must hot-water be applied to reach the most efficient and safe temperature for 100% quagga mussel mortality?
- For watercraft areas where spray water cannot be ≥54°C/130°F (i.e. ballast tanks and bladders); how long must the relatively cooler water be applied to reach the predetermined thresholds for 100% quagga mussel mortality?
- At what depths and time periods are the most quagga mussel pediveligers present between June 30, 2010 and December 28, 2010?
- At what depths and time periods does the most quagga mussel settlement occur between June 30, 2010 and December 28, 2010?

Hypotheses

H_{A1}: The tolerance of quagga mussels to hot-water with different temperatures is different and there is a difference between quagga mussels and zebra mussels in susceptibility to hot-water spray, and therefore must be treated with different temperatures and durations that ensure 100% mortality.

Quagga mussels are reported to have thinner shells than zebra mussels (Zhulidov et al. 2006), less tightly sealing shell valves (Claxton et al. 1997), and lower byssal thread synthesis rate in higher flows (Peyer et al. 2009). This means that quagga mussels may be more susceptible to hot-water sprays at lower temperatures than zebra mussels, and the application of hot-water spray to these two dreissenid species may be different.

H_{A2}: There is difference between the time it takes Category I areas and Category II areas of watercraft to reach the predetermined lethal temperature for quagga mussels.

The basis for this hypothesis is that Category I areas will be able to receive direct contact from the hot-water spray while Category II areas will not be able to. This means that in order for Category II areas to reach the necessary lethal temperature, more time must be taken to heat the total area to the specified temperature.

H_{A3}: For areas where spray water cannot be >54°C/130°F (Category III areas), there is a difference in the amount of time needed to reach the relatively cooler lethal temperature when compared to Category I areas.

The basis for this hypothesis is that Category III areas will have to receive hot-water spray at temperatures different from those necessary for the decontamination of Category I areas, and are therefore expected to take a different amount of time to achieve the amount of time necessary to ensure 100% quagga mussel mortality.

H_{A4}: There is a difference in the abundance of each stage of quagga mussel veligers present at different depths between the months of June, July, August, September, October, November, and December.

The basis for this hypothesis comes from previous research that noted that spawning of zebra mussels occurred in water with temperatures above 12°C (Sprung 1989). Since the average water temperatures of Lake Mead is greater than 12°C, it has been proposed that the quagga mussel may have multiple spawning cycles throughout the year (Gerstenberger et al. 2011) This would mean that there should be different concentrations and stages of quagga mussel veligers found throughout the year.

H_{A5}: There is a difference in the amount of quagga mussel colonization present at different depths between the months of June, July, August, September, October, November, and December.

The basis for this hypothesis comes from the proposed idea that quagga mussels may have various spawning cycles throughout the year if average water temperatures are greater than 12°C, which is a characteristic of Lake Mead (Gerstenberger et al. 2011). This would mean that competent pediveliger presence may be different between the months and the amount of settlement would vary.

Significance of the Study

This study was designed to provide valid field data on maintaining lethal temperatures for a minimum but sufficient amount of time necessary to achieve 100% quagga mussel mortality for watercraft and equipment decontamination in the western United States. The data can be used to modify and create standards for easily accessible areas, inaccessible areas, and areas with special temperature requirements of watercraft. The field data will also help policy makers in developing minimal thresholds for associated decontamination and inspection parameters. Data regarding the veliger abundance and colonization rates in Lake Mead will also provide valuable information to government agencies and policy makers for implementing anti-fouling measures in a timely and efficient way.

CHAPTER 2

REVIEW OF RELATED LITERATURE

Aquatic Invasive Species

Economic Impact and Spread

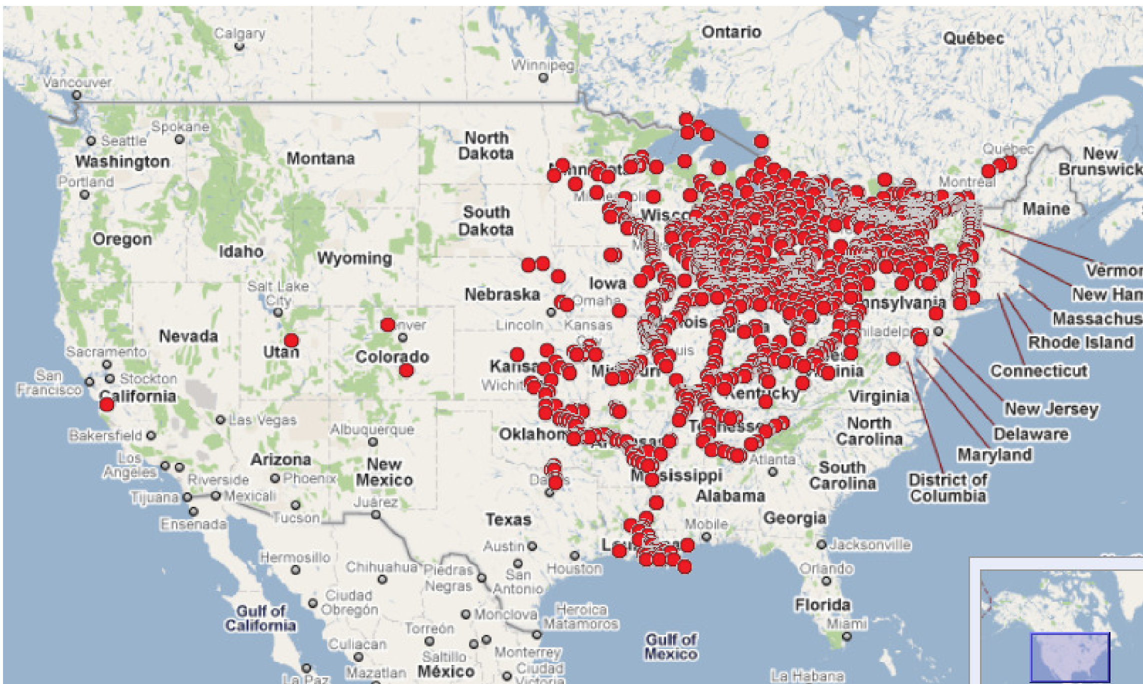
The establishment and subsequent invasion of certain non-indigenous species has proven to have profound negative economic, environmental, and even human health impacts (Keller 2007). In the United States alone, the cost and damages associated with aquatic invasive species (AIS) is estimated to be over $7 billion annually (Pimentel 2005). Many authorities believe that the ongoing spread of AIS to uncontaminated bodies of freshwater in North America can be ascribed to watercraft involuntarily transporting the AIS from a contaminated body of water (Bossenbroek et al. 2001; Johnson et al. 2001; Leung et al. 2006). While it is possible that some of the spread of AIS could be intentional, most cases of AIS translocation are most likely unintentional with the invasive organisms unknowingly present somewhere in or on the trailered vessel during overland transport (Johnson et al. 2001; Puth and Post 2005). There are many possible transport locations for AIS present on watercraft. These include undrained bait buckets, live wells, and bilge water, all of which provide favorable conditions for possible extended viability. They may also be present to some extent on the hull or entrained on boat exteriors, such as entangled on propellers and trailers (Rothelisberger 2010) or attached to other entangled organisms (Johnson et al. 2001). Any trailered vessel that makes contact with an AIS contaminated body of water should be treated as a potential vector for AIS.

Dreissenid Mussels

The zebra mussel (*Dreissena polymorpha)* and the quagga mussel *(Dreissena rostriformis bugensis),* commonly referred to as dreissenid mussels, are two of the most devastating AIS to invade North American freshwater systems (Western Regional Panel on Aquatic Nuisance Species 2010). The amazing fortitude and miniscule size of the two dreissenid species enables them to avoid discovery during watercraft inspections and remain viable during overland transport for several days, making them particularly important to accidental introduction to uncontaminated freshwater bodies (Ricciardi 1998).

Once established in a freshwater body, these mussels have been shown to clog water intake and delivery pipes, infest hydropower infrastructure, adhere to boats and pilings, foul recreational beaches, and cause many other financially costly problems (Western Regional Panel on Aquatic Nuisance Species 2010). Their introduction into the freshwater systems of the eastern United States (Figure 1b) have already lead to a multitude of ecological problems including competition with native mussels, disruption of food webs, and bioaccumulation of toxins (Wong 2011). The relatively recent discovery of quagga mussels in Lake Mead in January 2007 and their subsequent spread to the lower Colorado River system, as well as lakes and reservoirs in Arizona, California, Colorado and Utah (Figure 1a), resulted in the creation of several additional vectors for possible dreissenid spread. In order to properly understand methods to prevent further spread and management of these harmful AIS, it is important to understand the population dynamics and biology of the dreissenid mussel.

a) Quagga Mussel Distribution March 2011.



b) Zebra Mussel Distribution March 2011.

Figure 2. Dreissenid Distributions in North America (Benson 2011).

Quagga Mussel Biology

Life Cycle

The quagga mussel is a freshwater, bivalve mollusk that was originally native to the Dnieper and Bug River drainage systems in the Ukraine (Marsden et al. 1996). Analogous to other freshwater bivalves, quagga mussels have three main life cycle stages: larval veliger, juvenile, and adult stages (Crosier and Molloy 2001). Fertilization between the egg and the sperm occurs externally in the water column, and the larvae remain free-floating until the juvenile stage when they can settle and attach to a substrate with their proteinaceous byssal threads (Ackerman et al. 1994). The planktonic larval stage is divided into four separate stages: trochophore, straight-hinged veliger (also known as the D-shaped veliger), umbonal veliger, and pediveliger (Nichols and Black 1993). Embryological development of the fertilized egg directly results in the trochophore stage of the mussel. The organism is circular and 57-121 μm in diameter (Ackerman et al. 1994). The velum, a ciliated organelle, develops during this first stage, allowing the organism to move freely (Ackerman et al. 1994; Crosier and Molloy 2001). The next stage results when the larvae secrete an unornamented straight-hinged or D-shape shell two to nine days after fertilization (Crosier and Molloy 2001). The third stage occurs when the mantle tissue secretes an ornamented shell on the umbonal region near the hinges of the mussel seven to nine days after fertilization (Crosier and Molloy 2001). This umbonal stage is the last obligate free-swimming planktonic stage of the mussel (Ackerman et al. 1994). The last larval stage is the pediveliger which can swim using its velum or crawl by means of its foot, and secrete proteinaceous byssal threads that allow it to attach to a substrate and settle (Ackerman et al. 1994). After the pediveliger settles on

a substrate, it goes through metamorphosis to become a juvenile. Depending on certain environmental conditions (e.g. temperature, available substrates, food quantity), the amount of time necessary for a fertilized gamete to develop into a completely developed juvenile can range greatly, from 8 to 240 days (Nichols 1996).

Morphological Differences between the Quagga Mussel and the Zebra Mussel

Although the quagga mussel and zebra mussel are similar in many respects, they are distinguishable from each other by certain external morphological differences. One of the most evident characteristics has to do with the ventral surfaces of the two mussels. Zebra mussels have a flattened or slightly arched ventral surface that allows the shell to stand more-or-less upright when placed on a flat surface. Conversely, the ventral margin of the quagga mussel shell is typically convex and will lean when placed on a flat surface (Pathy 1993). Furthermore, the ventral line of the quagga mussel is generally curved while the zebra mussel has a more linear ventral line (Figure 3) (Domm et al. 1993). Additionally, quagga mussels have been reported to have thinner shells (Zhulidov et al., 2006), less tightly sealing shell valves (Claxton et al. 1997), and lower byssal thread synthesis rate in higher flows (Peyer et al. 2009) than zebra mussels.

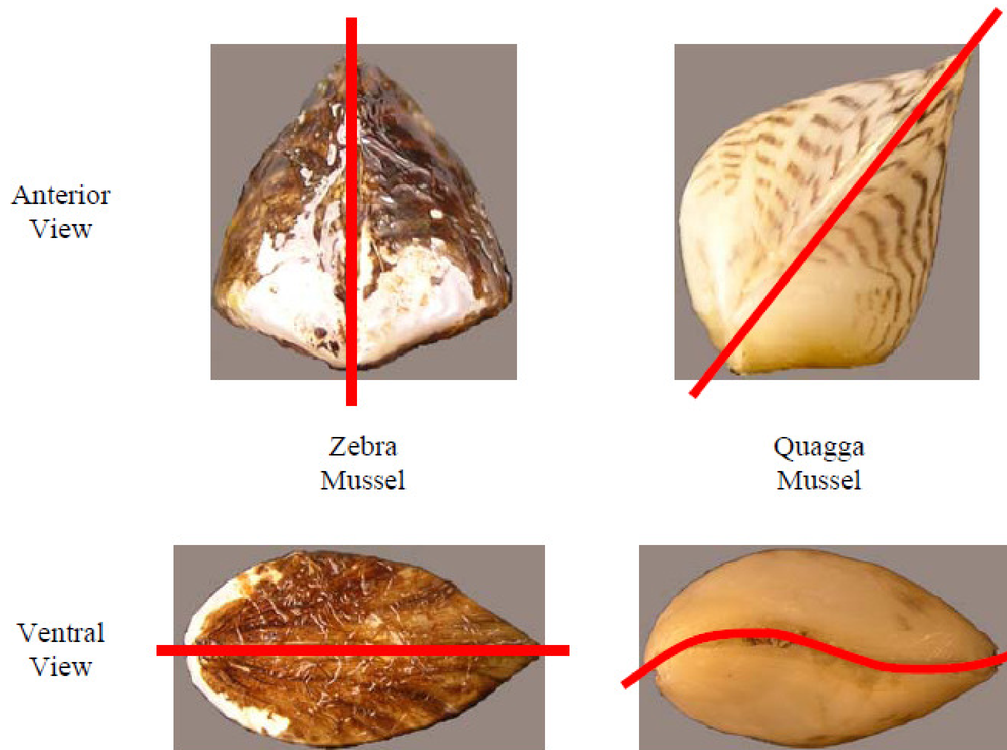


Figure 3. External morphology of the zebra mussel and the quagga mussel (Western Regional Panel on Aquatic Nuisance Species 2010).

Containment of Existing Dreissenid Populations

Watercraft Decontamination

The recent discovery and ensuing spread of dreissenid mussels to several previously uncontaminated inland bodies of water in the western United States (Benson 2010) has caused many federal, state, regional, and local agencies to initiate watercraft interception programs to prevent further infestations from occurring by implementing watercraft decontamination protocols (Zook and Phillips 2009). The objective of decontamination is to kill and remove all mussels (any stage of development) present on or in the watercraft. Ensuring all mussels are dead on the watercraft prevents the involuntary spread of the AIS to previously uncontaminated bodies of water. Making sure all mussels are removed

from the boat is also an important facet of decontamination because a false positive finding in an uncontaminated body of water may result from the presence of mussel shells (or DNA in samples collected for genetic analysis), even if the mussels were already dead (Zook and Phillips 2009). This can potentially cause unnecessary alarm and costly financial measures to be taken if unexplained shells drop or are scrapped off the hull and are then found at a boat ramp or at the lake bottom (Zook and Phillips 2009). Additionally, mussels which are present on watercraft that may appear to be dead may not necessarily be dead. There are some instances where the mussels may not be able to be completely removed from the watercraft such as in inaccessible areas (i.e. gimbal units); in these cases decontamination methods that ensure 100% mortality of the mussels present must be utilized to guarantee the AIS will not be spread. There are currently several accepted methods of watercraft decontamination which are currently approved by the United States Bureau of Reclamation (USBR). These include: chemical decontamination, heat, hot-water/high-pressure washing, freezing, physical removal, and desiccation (USBR 2010).

Chemical Decontamination

Quagga mussels and zebra mussels are resilient organisms which are capable of closing up and surviving for extended periods of time under external toxic conditions. For this reason, the successful use of chemical decontamination depends on the mussel life stage, the decontamination chemical used, and contact time (USBR 2010). Fully developed adult mussels may require as long as 10 days of contact time to ensure mortality. Consequently, chemical treatment is usually better suited to the veliger stage (immature life stage) of the mussel. Decontamination chemicals are relatively difficult to

use and successful results can be difficult to achieve. Some commonly used decontamination chemicals are: (1) one percent solution of table salt for 24 hours of contact time, (2) undiluted white vinegar for 20 minutes of contact time, (3) a diluted household bleach solution (> 5% sodium hypochlorite at a concentration of 3 ounces of bleach into 5 gallons of water) for a minimum of 1 hour, (4) potassium permanganate solutions, or (5) various quaternary ammonium and poly-quaternary ammonium compounds (USBR 2010). Some of these solutions may cause corrosion on metal surfaces and electrical connections, and may pose safety issues to the user. Therefore, guidelines about the proper use and disposal of decontamination chemicals should be followed closely.

Heat

Most authorities generally regard heat applications as the most efficient and easy to use of the decontamination methods. The temperature and exposure times determine the efficacy of the temperature treatments. Autoclaving, live steam, and boiling are all believed to be 100% effective at killing all dreissenid life stages. The water temperature to be used during hot-water washing or rinsing must be maintained at 140°F/60°C at surface contact for 1-3 minutes of exposure time to bring the surface temperature to the necessary 140°F/60°C for 30 seconds. In order to verify that this temperature has been achieved, a hand-held infrared temperature gauge should be used (USBR 2010)

Hot-water, High-pressure Washing

The use of hot-water, high-pressure washing is the most widely accepted method of watercraft mussel decontamination because of the ideal combination of lethal temperature water (at least 140°F/60°C), and the mechanical action of the high pressure to remove the

mussels from the watercraft. The United States Bureau of Reclamation (2010) recommends the following measures:

- Use a power washer unit that is capable of applying a flow rate of at least 4 gallons per minute with a nozzle pressure of 3,000 psi, and that is capable of supplying water at140°F/60°C or hotter at the surface point of contact.
- Begin the cleaning process by reducing the nozzle pressure by adjusting the power washer or using reduced pressure attachments. Do not attempt to remove or detach the mussels from the watercraft using high water pressure at this point in the cleaning process. The goal is to kill adult mussels with hot water while they remain attached to the surface.
- Rinse the entire area to be treated with heated water for at least 30 seconds of exposure time at 140°F/60°C to ensure 100% of all dreissenid mussel life stages. Depending on the size of the working area and the material composition of the surface, the operator may have to spray the surface for 1-3 minutes to achieve the necessary surface temperature.
- After rinsing the surface at reduced water pressure and achieving a surface temperature of at least 140°F/60°C for 30 seconds, maintain a hot-water temperature and increase the nozzle pressure high enough to detach the mussels from the surface.
- Continue treatment on all exposed surfaces of the watercraft.

Freezing

Adult zebra mussels have been shown to have a somewhat low tolerance to freezing temperatures. One study reported that 100% mortality occurred when individual mussels

were exposed to 14°F for as little as 1.3 hours (McMahon et al. 1993). The mussels do seem to have some more resistance to the cold when found in clusters, increasing the corresponding freezing mortality time at 14°F to be 4 hours for the most protected mussels (McMahon et al. 1993).

Physical

Physical crushing of mussels is an effective way to kill individual adult mussels, but it is not effective against the planktonic veliger stage or the small attached juvenile stage. It is relatively unpractical to use over a large area, and any crushed adult should also be exposed to a hot-water soak treatment prior to final dispose to ensure mortality (USBR 2010).

Desiccation

Desiccation is 100% effective at killing dreissenid mussels if sufficient time is allowed. Drying times capable of killing mussels can vary immensely according to the month of the year, location, and relative humidity; consequently, no single drying time estimate can ensure a complete 100% mortality for all situations, unless a set maximum time is used. In some cool and highly humid settings, it is estimated that mussels can survive for over 40 days out of water (USBR 2010). For up-to-date specific information regarding desiccation time for a given month, location, and prevailing conditions, refer to the 100th Meridian *Quarantine Estimator for Zebra Mussel Contaminated Boats* drying schedule at the following Web site: http://www.100thmeridian.org/Emersion.asp. (100th Meridian Initiative 2011)

CHAPTER 3

METHODOLOGY

Hot-Water Spray Decontamination of Quagga Mussels

The use of hot-water spray as a method of watercraft decontamination for dreissenid mussels is widely accepted by many federal agencies. These agencies most commonly decontaminate watercraft with pressurized hot-water spray temperatures exceeding 60°C. This temperature is based on acute (short-term) upper-thermal-limit data generated for spray for mitigation of immersed mussels. The first study regarding the use of hot-water spray for mitigation of emersed zebra mussels fouling, which is closely related to a field situation where sprays are applied to watercraft, was by Morse (2009). Morse found that the survivorship of mussels was affected by two major factors: spray water temperature and exposure duration. Water sprayed at ≥60°C for 10 seconds or 80°C at ≥5 seconds was shown to be 100% lethal to zebra mussels. This indicates that a decontamination recommendation of spray temperatures of ≥60°C may not result in 100% mortality if the exposure duration is less than 10 seconds (Morse 2009). The information from Morse (2009) is helpful in generating and revising uniform minimum protocols and standards for watercraft interception programs (Zook and Phillips 2009).

Morse's (2009) findings are quite useful because it was the first study to test thermal spray treatments on emersed mussels and as such, provides a solid starting point for determining effective field application for watercraft decontamination. There are, however, several important aspects which needed to be addressed regarding species-specific application. This is a key component because some inland bodies of water may be infested with only zebra mussels, quagga mussels, or both. In the western United

States, quagga mussels are of particular importance, as they are currently the most widespread dreissenid species, whereas only one water body in California is infested by zebra mussels (Benson 2010). Previous studies have shown that there are differences between these two dreissenid species (Pathy 1993; Ricciardi 1994; Mills 1996; Balwin 2002; Peyer et al. 2009), and it is important to determine if the quagga mussel is more or less susceptible than the zebra mussel to hot-water spray. Studies have also shown that the upper thermal limit of the quagga mussel is lower than that of the zebra mussel (Mills et al. 1996). Zebra mussels survive indefinitely at 30ºC, but quagga mussels show rapid mortality at 30ºC (Spidle et al. 1995; McMahon 1996). Quagga mussels are also reported to have thinner shells (Zhulidov et al. 2006), less tightly sealing shell valves (Claxton et al. 1997), and lower byssal thread synthesis rates in higher flows (Peyer et al. 2009). Therefore, quagga mussels may be more susceptible to death by hot-water sprays at a lower temperature than zebra mussels, and the application of hot-water spray to these two dreissenid species may be different.

To be effective and efficient in mitigating biofouling by invasive quagga mussels in the western United States, hot-water spray thresholds needed to be evaluated specifically for quagga mussels. In order to accurately determine the temperatures and exposure times necessary to attain 100% mortality of specimens of quagga mussels following exposure to a hot-water spray, the present study investigated the lethal effect of hot-water sprays on emersed specimens of quagga mussels at water temperatures ranging from 20ºC to 80ºC and exposure durations of 1, 2, 5, 10, 20, 40, 80, and 160 s. The field data was then compared to existing data regarding zebra mussels to determine if there is any difference in susceptibility regarding the two dreissenid species. The data was also used in an

evaluation of the necessary time needed to reach and sustain the lethal temperatures in inaccessible areas (Category II) and heat-sensitive areas (Category III), respectively.

Field Tests on Emersed Quagga Mussels

Specimen Collection and Holding Conditions

Specimens of adult *D. rostriformis bugensis* (≥12mm in length) were collected from the hull of an encrusted National Park Service boat which was stationed in Lake Mead, Nevada-Arizona, USA. The individuals were then divided among 60 mesh spat bags (~75 in each) and acclimated to the lake water in a boat slip within the Las Vegas Bay Marina (N 36º01.764, W 114º46.400) for two weeks prior to experimentation.

Experimental Design and Measurements

After acclimation, adult mussels were randomly divided into 60 subsamples (n = 50) and placed into 60 identical pre-labeled 3.0 mm spat bags (Aquatic Eco-Systems Inc., Apopka, FL) (Table 2). Each bag was then suspended over one of two, identical open Polyscience Programmable heated circulator wash baths with a 28 liter capacity during the thermal spray treatment (VWR International Inc.). The purpose of using two water baths was to increase the efficiency and speed at which the tests could be conducted by allowing limited water temperature variation. Each mesh spat bag containing a test subsample was held horizontally 20 cm over the heated water bath to prevent any difference in ambient air temperature which may have resulted from the heated water in the open water baths. Treatment spray was then applied to the samples at a flow rate of approximately 900 ml min^{-1} through a fan shaped nozzle**.** The distance above each sample at which the spray was applied was modified each time in order to maintain the constant test temperature used for each specific subset. This was done because the

environmental field conditions, i.e. wind, rain, ambient air temperature, would affect the contact water temperature if there was a set distance. The specific distances prior to each spray were determined using a ruler and a fast-reacting remote water temperature probe (Pace Scientific Model XR440 Pocket Logger with 4 temperature probes). The distance between the spray nozzle and the contact point of the water at the necessary test temperature was then calculated. The Pace Scientific Model XR440 Pocket Logger was calibrated prior to use and an NIST traceable certificate of validation was included from the manufacturer. Temperature readings obtained from the temperature probes were also verified by the use of a Raytek MT4 non-contact mini infrared thermometer. The thermal spray was immediately applied to the specific subset at the specifically calculated distance. Each subset of mussels was positioned within the spat bag to form a horizontal line not exceeding 5 cm in width in order to allow the hot-water spray to be equally distributed over all of the mussels. The polyethylene mesh of the spat bags allowed the water spray to pass over them without additional pooling or heat transfer beyond that would normally occur from direct exposure to the spray (Morse 2009). Each sample of mussels was separately exposed to thermal-spray treatments at 20, 40, 50, 54, 60, 70, and 80°C and exposure durations of 1, 2, 5, 10, 20, 40, 80, and 160 s. Therefore, 56 combinations on temperature by exposure duration were treated (Table 2). Four bags which were not treated with hot-water spray were used as controls.

Following treatment, each spat bag containing the treatment specimens was then attached to one of the seven 1 cm braided nylon lines (one for each temperature set) spanning the boat slip. These lines were attached to a grid composed of ABS pipe which was positioned on either side of the slip to allow easy access to the samples. Each line

holding the spat bags was approximately 1.5 m out of the water and the mussels within the bags were kept at a depth of approximately 2 m.

Table 2. Amount of Adult Quagga Mussels Tested per Treatment Group (n = 50 per group).

Temperature		1 s	2 s	5 s	10 s	20 s	40 s	80 s	160 s
°F	°C								
68	20	50	50	50	50	50	50	50	50
104	40	50	50	50	50	50	50	50	50
122	50	50	50	50	50	50	50	50	50
130	54	50	50	50	50	50	50	50	50
140	60	50	50	50	50	50	50	50	50
158	70	50	50	50	50	50	50	50	50
176	80	50	50	50	50	50	50	50	50

Data Collection and Management

Sample mortality was then recorded immediately after testing and daily thereafter for 10 days. The viability was tested by inspecting post-treatment samples for specimens with widely gaping valves, similar to the kind conducted by Morse (2009). The bags containing specimens were individually removed from the water and examined on a plastic table. The mussels were gently prodded on their shell valves with a pair of blunt-end forceps. Specimens who did not respond by immediate shell valve closure were then gently stimulated in the area of their inhalant and exhalent siphons using slight pressure from fingertips. Those which did not respond to this latter stimulus by immediate valve closure had their shell valves forcibly closed by pressure from the fingertips. If their valves immediately re-opened after release from the fingers, specimens were considered to be dead (Morse 2009). The dead mussels were then completely opened using pressure from fingers to ensure that they would continue to be counted as dead. At the end of the

10 day period, the total mortality for each of the groups was determined and the shell lengths of each mussel (i.e. the greatest distance from the anterior tip of the umbos to the posterior shell valve margins measured to the nearest 0.01 mm with digital calipers) was recorded. The control group samples (n = 50 each) were continuously immersed in the lake over the same 10 day period and recovery period. Their survivorship was assessed daily as described above. The data was stored in the Environmental Science Laboratory at UNLV.

Statistics and Data Analysis

A two-way analysis of variance (ANOVA) was used to exam if there was any significant difference in shell length at different temperatures with different exposure durations, and an independent samples t-test was used to determine if there was any significant difference in shell length between mussels in the treatment group (pooled data) and the control group (Zar 1996). The significance criterion was set at $\alpha = 0.05$. A binary logistic regression model was used to estimate mortality (a binary response) as a function of exposure time and water spray temperature. Model parameters and their associated standard errors were used to produce estimates and confidence intervals of the LT_{50} and LT_{99} values, and these estimates were further used to compare the corresponding LT_{50} and LT_{99} values for zebra mussels generated by Morse (2009). LT_{50} and LT_{99} estimates were defined as the temperatures required to induce sample mortalities of 50% and 99%, respectively. All the statistics and model estimation were performed using SAS® (Version 9.2, SAS Institute Inc. Cary, NC).

Evaluation of Category II Watercraft areas

For areas of the watercraft that are not directly exposed to hot-water sprays, the time necessary to pre-heat these locations to the lethal thermal temperature was evaluated because heat loss can occur during the water flow to these areas. The gimbal unit was tested on an uncontaminated boat and the contact temperature (internal temperature) was monitored until it reached the lethal temperature. The temperature of the water exiting the gimbal unit was monitored by use of a fast-reacting remote water temperature probe (Pace Scientific Model XR440 Pocket Logger with 4 temperature probes). Temperature readings from the contact water at the temperature probes were verified by the use of a Raytek MT4 non-contact mini infrared thermometer. The data from this test can be applied to Category II decontamination areas (Table 1). Since weather conditions, especially ambient temperature, could be a confounding factor affecting the surface temperature of these areas, the experiment was conducted twice, once in winter and again in the summer.

Evaluation of Category III Watercraft Areas

In some areas of watercrafts, the temperature cannot exceed 130°F (Zook and Phillips 2009). Therefore, the necessary time it takes to reach and maintain a lethal water temperature needed to be evaluated for these areas. The contact temperature (internal temperature) was monitored on the live wells and bait wells of an uncontaminated boat until it reached the lethal temperature. The temperature of the water exiting these areas was monitored by use of a fast-reacting remote water temperature probe (Pace Scientific Model XR440 Pocket Logger with 4 temperature probes). Temperature readings from the contact water at the temperature probes were verified by the use of a Raytek MT4 non-

contact mini infrared thermometer. Since weather conditions, especially ambient temperature, could be a confounding factor, this experiment was conducted twice: once in winter and again in the summer. The time data from this test can be applied to Category III (Table 1) decontamination areas.

Summer and Winter Validation

After the minimum amount of time necessary to achieve the predetermined lethal temperature at Category I and Category II areas was determined from the aforementioned experiments, the data was tested on actual boats encrusted with quagga mussels for both the winter and summer. All hot-water treatments were applied using a portable hot-water watercraft decontamination system (Hydroblaster GHO-MDS, Hydro Engineering, Inc., Salt Lake City, UT), and the temperatures were assessed by use of a fast-reacting remote water temperature probe (Pace Scientific Model XR440 Pocket Logger with 4 temperature probes). Temperature readings from the contact water at the temperature probes were verified by the use of a Raytek MT4 non-contact mini infrared thermometer. For the treatments, at least six samples from Category I areas were tested and at least two samples from Category II areas. Control groups were also taken from the specific areas of the boat before hot-water spray treatment. After each treatment for each category under different conditions, the treatment samples were transferred to individually labeled 3 mm mesh spat bags and submerged in Lake Mead. Dead mussels were identified and mortality counts were taken 10 days after treatment (Comeau et al. 2011). The additional untreated control groups of mussels were also immersed continuously in the lake for 10 days and had their mortality counts evaluated. The mortality rate for different treatment conditions was then calculated.

Veliger Collection and Substrate Colonization

Method of Veliger Collection and Analysis

Every week from June 30, 2010 until December 28, 2010, a vertical plankton tow (mesh size of 63 µm) was used to collect veliger samples in the open water near Sentinel Island, Lake Mead. The location was marked by two emerged orange buoys. The GPS coordinates of the buoys was N 36º03’13’’ W114 º44’58’’. Samples were collected by gently lowering plankton net to the water at rate of approximately 1 m/second and pulling the net back up from depths of 5, 10, 20, 30, 40, 50 and 60 m. The net was pulled up relatively slowly seeing as pulling too fast can create a pressure wave in front of the net that pushes the water and plankton away from the mouth of the net, and therefore, may not effectively sample the desired volume of water. After each tow, the net was washed top to bottom from the outside with distilled water to rinse veligers into the collection cup. After the proper amount of tows for the certain depth were performed, the collection cup side screens were also washed from top to bottom and then emptied into a 500 mL Nalgene bottle. The collection cup was then rinsed twice with small amounts of deionized (DI) water and emptied into the same bottle. Each bottle was labeled with the date and depth. Sample bottles were kept on ice while in the field. Once sampling was complete each week, the plankton net was thoroughly rinsed with clean water. The sample was preserved in the field using ethyl alcohol at 25% of the final sample volume. Samples were refrigerated until they were analyzed.

Table 3. Sampling Volume Calculated per Tow at Specific Depths.

	Depth (m)						
Collection Net Diameter = 0.20 m	5	10	20	30	40	50	60
How many tows	7	4	2	1	1	1	1
Volume after 1 tow (L)	157	314	628	942	1257	1571	1885
Total Volume (L) after tows*	1099	1256	1256	942	1257	1571	1885
* Rinsing volume is not counted							

Quagga mussel veligers were processed and counted in the laboratory using a modified combination of the Standard Method (10200 G) Zooplankton Counting Techniques (Eaton et al. 2005), the US. Army Corps of Engineers (USACE) method for calculating *Dreissena* veliger densities, and the NALGENE™ Imhoff settling cone instructions. In the laboratory, the each sample was added to an Imhoff settling cone with a venoset delivery system. The veligers were allowed to settle in the Imhoff cone for 45 minutes, the sides were then gently stirred with a glass rod and the sample was allowed to sit for an additional 15 minutes. Aliquots of the settled sample were transferred into a centrifuge tube (50 mL). One mL of the well-mixed sample was then pipetted and dispensed into a Sedgwick-Rafter counting cell. A cover slip was then placed on the counting cell perpendicular to the long axis of the slide. The filled Sedgwick-Rafter cell was examined under a dissecting microscope fitted with a cross-polarized light (Carl Zeiss SteREO Discovery.V8, Toronto, Ontario, Canada). The utilization of cross-polarization of the light aided in counting veligers due to the birefringent crystalline structure of the calcite in their larval shell (Johnson 1995). The size of veligers in the sample were measured with the AxioVision 4 Image Anaysis Software set up for an

AxioCam (Carl Zeiss Inc.) which connected a computer to the stereomicroscope in order to aid in determining the specific stage of development.

The size of a veliger refers to the length measured perpendicular to the axis from the umbo or center of the hinge line to the opposing margin of the shell (Nichols and Black 1994). The percent proportion of each veliger developmental stage was recorded for each week at each specific depth. This is a recommended standard monitoring protocol for veliger monitoring in the Lower Colorado Region (Wong et al. 2011). Different developmental stages of veligers were identified based on the keys provided by Nichols and Black (1994). Trochophore stage veligers were typically circular with a larval shell present, and were <110 μm in size. D-shaped veligers were found to have a straight hinge line (i.e. d-shaped shell), and were typically 111-140 μm in size. Umbonal stage veligers were found to develop an umbo (bump), but it was typically low and rounded. Umbonal stage veligers were typically 141-210 μm in size. Pediveliger stage veligers had a pronounced, knobby umbo present, and parts of the right valve margin extend beyond the margin of the left. Pediveligers were typically ≥211 μm in size.

<u>Method of Substrate Collection and Analysis</u>

Every two weeks, from July 2010 until December 2010, a 2 × 2 inch fiberglass substrate plate was placed at depths of approximately 5, 10, 20, 30, and 40 m in the open water near Sentinel Island, Lake Mead. The location was marked by two emerged orange buoys. The GPS coordinates of the buoys was N 36º03’13’’ W114 º44’58’’. The plates were suspended in the water on a 50 m nylon rope at the buoy location at each depth interval and affixed to the rope vertically by means of zip-ties. The end of the rope had a steel chain tied to the end to prevent the hanging rope from drifting with the current and

becoming entangled with the buoys. The plates were exchanged biweekly and stored in a Petri dish sealed with tape in the field. The edges of each plate were wiped clean before being placed in the Petri dish to ensure that only the settlers on the two faces of the slide were counted. The Petri dishes containing the slides were stored on ice in the field and transferred to a refrigerator until they were analyzed.

Quagga mussel settlers on the plates were analyzed in the laboratory using a dissecting microscope fitted with a cross-polarized light (Carl Zeiss SteREO Discovery.V8, Toronto, Ontario, Canada). Each substrate plate was rinsed with DI water within the Petri dish it was kept in and gently had each face of the slide scraped to ensure all settlers were counted.

CHAPTER 4

FINDINGS OF THE STUDY

Results from Field Tests on Emersed Mussels

After analysis of the data, it was found that there was a trend which indicated that the higher temperatures induced greater mortality following the same exposure duration (Figure 4, Table 4). Spray exposures of 1 s or 2 s were not found to induce 100% mortality at any of the test temperatures (Table 4). However, a 5 s spray exposure did result in 100% mortality (≥60°C). The other temperature and time combinations that resulted in 100% mortality were 54°C for 10 s, 50°C for 20 s, and 40°C for 40 s. Estimated LT_{50} values for 1 s, 2 s, and 5 s indicate that the temperature to kill 50% of the mussels was between 47.2°C to 47.9°C (Table 5), while the estimated LT_{99} with these exposure durations varied significantly from >80°C at 1 s and 2 s to 58.8°C at 5 s (Table 5).

Table 4. Quagga Mussel Mortality (%) under Different Treatments at Day 10.

Temperature (°C)	1 s	2 s	5 s	10 s	20 s	40 s	80 s	160 s
20	4%	4%	6%	0%	0%	2%	2%	0%
40	2%	2%	8%	12%	94%	100%	100%	100%
50	10%	22%	36%	82%	100%	100%	100%	100%
54	54%	72%	98%	100%	100%	100%	100%	100%
60	72%	92%	100%	100%	100%	100%	100%	100%
70	88%	98%	100%	100%	100%	100%	100%	100%
80	86%	98%	100%	100%	100%	100%	100%	100%

Note: The mortality of control (n = 4) was 3%.

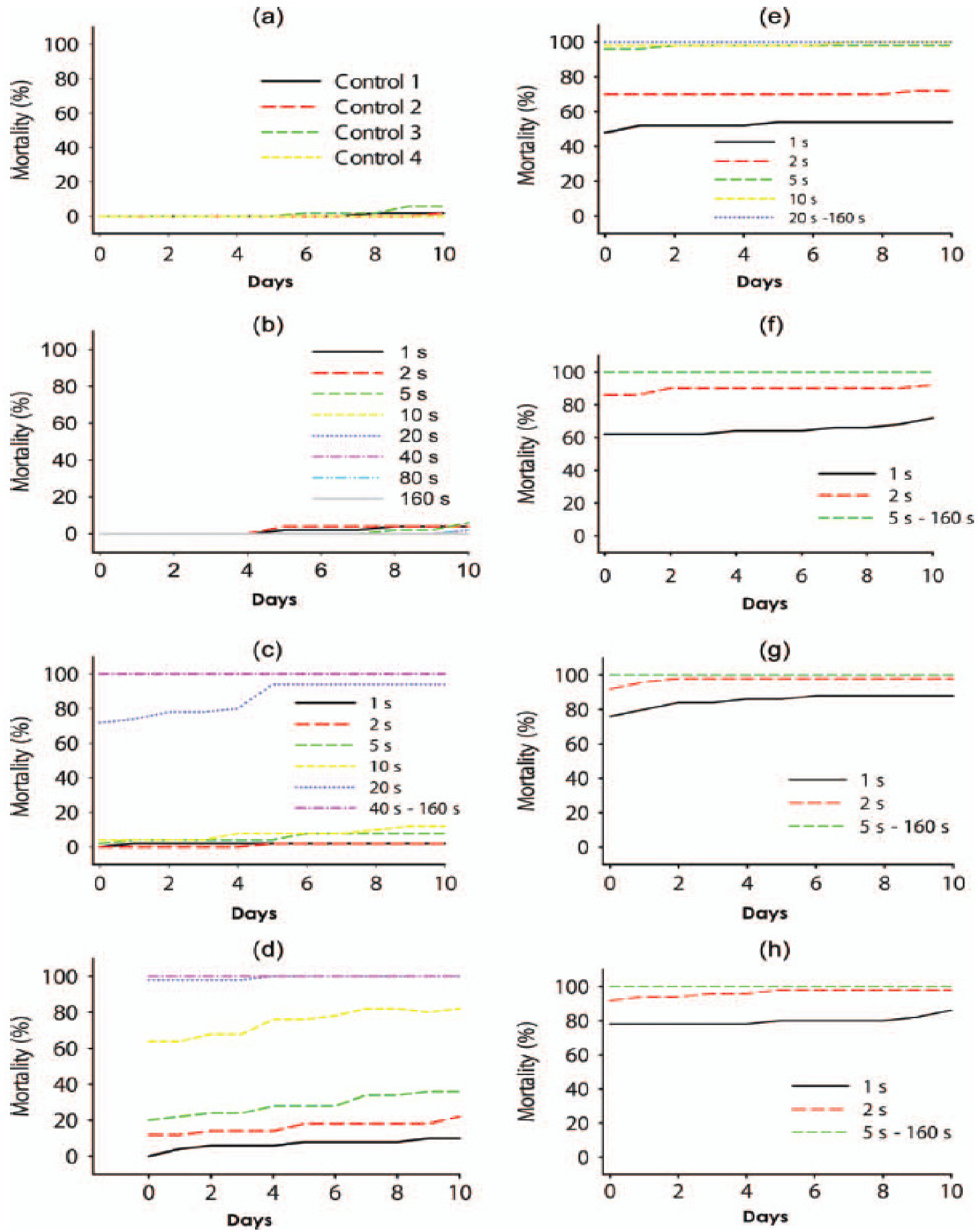


Figure 4. Mortality (%) of Quagga Mussels in Lake Mead after Hot-Water Spray Treatment. (a) Control (11.86°C); (b) 20°C; (c) 40°C; (d) 50°C; (e) 54°C; (f) 60°C; (g) 70°C; (h) 80°C. Note that (c) and (d) share the same symbol and line styles. (Comeau et al. 2011).

The continuously immersed control samples (11.86°C ± 1.60) and the samples exposed to the 20°C spray treatments exhibited high survival rates over the 10 day period. The combined four groups of controls exhibited 97% survival (ranging from 94% to 100% (Figure 4a)), and the eight 20°C spray treatment subsamples displayed a mean 98% survival rate (ranging from 94% to 100%) with no apparent correlation to duration time (Figure 4b). Survival was also high for 40°C at spray exposures of 1 s (98% survival), 2 s (98% survival), 5 s (92% survival), 10 s (88% survival), and for 50°C at 1 s (90% survival).

Table 5. Estimated LT_{50} and LT_{99} Values (in bold) and their 95% Confidence Limit for Hot-Water Spray Treatments on Quagga Mussels at 1 s, 2 s, and 5 s Application Durations (*n* = 350 for each duration).

Duration (s)	LT_{50} (°C)	LT_{99} (°C)	SM_{100} (°C)*
1	44.1 < **47.9** < 52.5	> 80	> 80
2	44.0 < **47.8** < 52.3	> 80	> 80
5	43.5 < **47.2** < 51.7	54.1 < **58.8** < 64.4	60

*The SM_{100} is the temperature observed in the experiment that induced 100% mortality

The average shell length for the hot-water spray treated mussels ranged from 18.65 mm to 20.00 mm (Table 6). Shell length of mussels in the 56 treatment groups (mean = 19.2 mm, range = 18.7 - 20.0 mm, Table 1) did not differ significantly between temperature and exposure duration combinations (Two-way ANOVA, DF = 13, F = 1.5, P = 0.1), and was comparable (T-test, DF = 2998, t = -0.29, P = 0.77) to the controls (mean = 19.0 mm).

Table 6. Shell Length (mm) of Quagga Mussels for the Hot-Water Spray Experiment (n = 50 for each combination of temperature and exposure duration).

Temperature (°C)	1 s	2 s	5 s	10 s	20 s	40 s	80 s	160s
20	19.64 ± 2.44	19.44 ± 2.30	19.00 ± 2.01	19.53 ± 2.24	19.38 ± 2.10	19.32 ± 2.23	19.52 ± 2.22	19.69 ± 2.26
40	19.82 ± 2.50	19.29 ± 2.18	18.94 ± 2.26	19.23 ± 2.49	19.30 ± 2.48	19.21 ± 2.17	19.41 ± 1.94	19.02 ± 1.90
50	19.71 ± 2.14	19.93 ± 2.25	19.84 ± 2.04	19.22 ± 2.03	19.14 ± 2.20	19.64 ± 2.47	20.00 ± 2.54	19.30 ± 2.00
54	18.77 ± 2.18	18.77 ± 2.31	19.27 ± 2.50	19.89 ± 1.88	19.40 ± 2.03	19.56 ± 2.55	18.90 ± 2.56	19.20 ± 2.08
60	19.21 ± 2.30	18.84 ± 2.41	19.33 ± 2.52	19.03 ± 2.03	19.01 ± 2.47	19.37 ± 2.04	19.60 ± 1.98	19.64 ± 2.28
70	19.69 ± 2.90	19.53 ± 2.52	18.57 ± 2.97	18.91 ± 2.38	19.34 ± 2.17	18.86 ± 2.36	19.33 ± 2.08	18.65 ± 2.43
80	19.71 ± 2.71	18.86 ± 2.38	18.68 ± 2.34	19.15 ± 2.45	19.24 ± 2.08	19.35 ± 2.48	19.72 ± 2.05	19.88 ± 2.56

Note: The shell length (mm) of the four control groups are 19.50 ± 2.13, 19.08 ± 1.95, 19.1 ± 1.81, and 19.32 ± 2.13, respectively.

Results from the Evaluation of Category II areas

As expected, there was an increase in time needed to reach and sustain the lethal hot-water temperature in the tested Category II area (the gimbal unit). There was also an increase noticed in the amount of time necessary to achieve the predetermined temperature between the winter and summer conditions (Tables 7-8). The summer and winter evaluation experiments were conducted September 17, 2010 and January 21, 2011, respectively, on inboard/outboard Mercruiser engines.

Table 7. Evaluation of Lethal Temperature Recommendations on the Gimbal Unit during Summer Weather Conditions.

Hot Water Temp (°F)	Hot Water Temp (°C)	Air Temp (°F)	Air Temp (°C)	Target Temp (°F)	Target Temp (°C)	Attempt	Time to reach target temp (min)
140	60	95	35	140	60	1	0.42.9
140	60	96	35.6	140	60	2	0.42.0
140	60	98	36.7	140	60	3	0.37.1

Table 8. Evaluation of Lethal Temperature Recommendations on the Gimbal Unit during Winter Weather Conditions.

Hot Water Temp (°F)	Hot Water Temp (°C)	Air Temp (°F)	Air Temp (°C)	Target Temp (°F)	Target Temp (°C)	Attempt	Time to reach target temp (min)
140	60	39	3.9	140	60	1	02:06.0
140	60	41	5	140	60	2	02:06.8
140	60	43	6.1	140	60	3	01:58.3

Results from the Evaluation of Category III areas

For areas on watercrafts that cannot withstand temperatures above 130°F/54°C, flush tests with 54°C water were ran on the live and bait wells of a Cobia 296 boat. Just as in the evaluation of Category II areas, there were tests conducted during the winter and summer which showed a difference between the necessary application times in the differing ambient conditions (Tables 9-12). The summer and winter evaluation experiments were conducted September 1, 2010 and January 21, 2011, respectively.

Table 9. Evaluation of Lethal Temperature Recommendations on the Bait Wells during Summer Weather Conditions.

Hot Water Temp (°F)	Hot Water Temp (°C)	Air Temp (°F)	Air Temp (°C)	Target Temp (°F)	Target Temp (°C)	Attempt	Time to reach target temp (min)
130	54	98	36.7	130	54	1	00:33.1
130	54	98	36.7	130	54	2	00:34.4
130	54	98	36.7	130	54	3	00:33.9

Table 10. Evaluation of Lethal Temperature Recommendations on the Bait Wells during Winter Weather Conditions.

Hot Water Temp (°F)	Hot Water Temp (°C)	Air Temp (°F)	Air Temp (°C)	Target Temp (°F)	Target Temp (°C)	Attempt	Time to reach target temp (min)
130	54	38	3.3	130	54	1	01:06.9
130	54	38	3.3	130	54	2	01:05.4
130	54	39	3.9	130	54	3	01:03.1

Table 11. Evaluation of Lethal Temperature Recommendations on the Live Wells during Summer Weather Conditions.

Hot Water Temp (°F)	Hot Water Temp (°C)	Air Temp (°F)	Air Temp (°C)	Target Temp (°F)	Target Temp (°C)	Attempt	Time to reach target temp (min)
130	54	98	36.7	130	54	1	01:10.1
130	54	98	36.7	130	54	2	00:58.2
130	54	98	36.7	130	54	3	01:06.9

Table 12. Evaluation of Lethal Temperature Recommendations on the Live Wells during Winter Weather Conditions.

Hot Water Temp (°F)	Hot Water Temp (°C)	Air Temp (°F)	Air Temp (°C)	Target Temp (°F)	Target Temp (°C)	Attempt	Time to reach target temp (min)
130	54	38	3.3	130	54	1	01:51.4
130	54	38	3.3	130	54	2	01:41.9
130	54	38	3.3	130	54	3	01:20.5

Boat Decontamination Validation Results

In order to accurately determine if the results regarding the susceptibility of quagga mussels to hot-water spray from the previous experiments was applicable as a means of watercraft decontamination, it was necessary to conduct actual field experiments on watercrafts that were encrusted with quagga mussels. Category I areas were sprayed with hot-water at a temperature of 60°C for a duration of 5 s because it was the lowest temperature capable of 100% mortality at 5 s and higher temperatures could be seen a threat to human health (Morse 2009). Category II areas were also sprayed with hot-water at a temperature of 60°C, but used the longest duration determined from the evaluation of the areas depending on the season; 43 s for summer, and 2 minutes and 7 s for winter. There was a separate validation for both categories for summer and winter.

Summer Validation

The summer validation experiment took place on September 28, 2010 with an ambient air temperature averaging 95°F/35°C. There were seven replicates for the Category I assessment and three controls. These were located on various freely accessible areas on the boat and were sprayed with 60°C water for a duration of 5 s. For each of the

replicates, 100% mortality was achieved immediately after testing (Table 13). There were two replicates and one control for the Category II assessment which evaluated the encrusted gimbal unit of the watercraft. The gimbal unit was flushed with 60°C water for a duration of 48 s (including 5 s of duration to ensure the predetermined lethal duration was met). For each of the replicates, 100% mortality was achieved immediately after testing (Table 14). There was a significant difference in the percent mortality of the experimental groups and the controls for each of the tested categories.

Table 13. Number of mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category I Areas (summer).

Group	Number of mussels present	Number of mussels dead	Mortality	Average shell length (mm)
1	121	121	100%	7.28 ± 0.99
2	163	163	100%	8.07 ± 1.47
3	271	271	100%	8.04 ± 1.48
4	30	30	100%	6.77 ± 1.13
5	39	39	100%	4.99 ± 1.50
6	77	77	100%	5.62 ± 1.80
7	35	39	100%	5.25 ± 1.37
Control 1	126	4	3%	8.60 ± 1.84
Control 2	111	19	17%	8.15 ± 1.97
Control 3	146	8	6%	8.64 ± 1.85

Table 14. Number of mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category II Areas (summer).

Group	Number of mussels present	Number of mussels dead	Mortality	Average shell length (mm)
1	109	109	100%	7.12 ± 1.18
2	94	94	100%	7.92 ± 2.31
Control	57	18	32%	10.79 ± 2.83

Winter Validation

The winter validation experiment took place on January 27, 2011 with an ambient air temperature averaging 50°F/10°C. There were six replicates for the Category I assessment and three controls. These were located on various freely accessible areas on the boat and were sprayed with 60°C water for a duration of 5 s. For each of the replicates, mortality was assessed after 10 days of immersion in Lake Mead after treatment and 100% mortality was achieved for each replicate (Table 15). There were two replicates and one control for the Category II assessment which evaluated the encrusted gimbal unit of the watercraft. The gimbal unit was flushed with 60°C water for a duration of 2 minutes and 12 s (adding 5 s of duration to ensure the predetermined lethal duration was met). Only one replicate from the gimbal unit had a resulting 100% mortality, while the other exhibited 96% mortality (Table 16). There was a significant difference in the percent mortality of the experimental groups and the controls for each of the tested categories.

Table 15. Number of mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category I Areas (winter).

Group	Number of mussels present	Number of mussels dead	Mortality	Average shell length (mm)
1	55	55	100%	16.04 ± 4.02
2	43	43	100%	18.73 ± 3.85
3	48	48	100%	14.78 ± 3.55
4	34	34	100%	15.24 ± 4.93
5	64	64	100%	13.57 ± 3.25
6	37	37	100%	17.03 ± 4.58
Control 1	134	2	2%	12.59 ± 4.54
Control 2	107	0	0%	12.35 ± 4.78
Control 3	83	0	0%	12.35 ± 3.31

Table 16. Number of mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category II Areas (winter).

Group	Number of mussels present	Number of mussels dead	Mortality	Average shell length (mm)
1	77	77	100%	13.70 ± 3.07
2	55	53	96%	13.70 ± 3.73
Control	125	2	2%	13.12 ± 4.78

Results on Veliger Collection at Specific Depths in Lake Mead

Quagga mussel veligers were found to be present in the water column of Lake Mead consistently from June 30, 2010 to December 28, 2010. The concentration and abundance was found to vary throughout the six-month period and there was also a noticeable variation in abundance between the different measured depths. The largest peaks present for this period was found to be on August 18, 2010 and September 1, 2010 from 20 m tows which had a calculated abundance of 31.0 veligers per liter and 30.5 veligers per liter, respectively. It is interesting to note that a majority of the veligers were found to be present at depths between 10 m and 30 m. From the data collected, there was an increase of veliger abundance from July until mid-September 2010 which was followed by a gradual decrease till December 2010 (Figure 5).

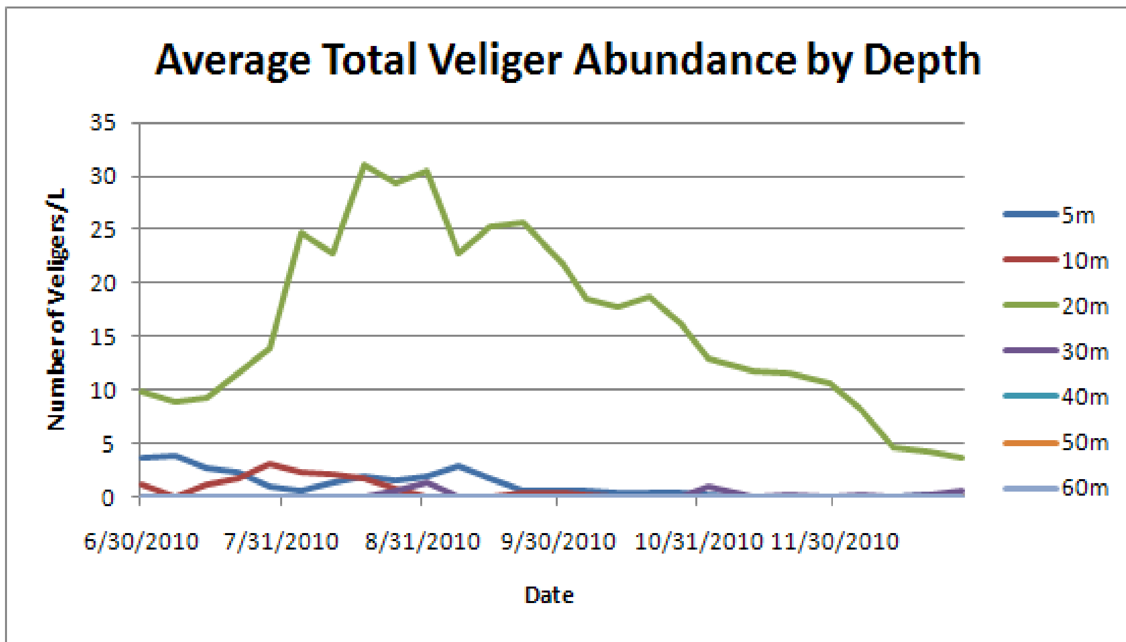


Figure 5. Total Veliger Abundance per L Calculated by Depth. Averages were taken weekly from 06/30/2010 to 12/28/2010.

There was a relatively low abundance of total veligers seen at depths of 5 m (peak at 3.9 veligers/L) and 10 m (peak at 4.8 veligers/L), and veligers at these depths were also seen to decrease in abundance throughout the period of the study (Figure 5). There was little veliger abundance at 30 m in depth, and virtually no abundance at depths of 40, 50, and 60 m.

It was found that the highest abundance of pediveligers occurred during the month of August 2010 (>10 pediveligers/L), and the lowest was found to be during October 2010 (<2 pediveligers/L) (Figure 6). For depths of 5 m and 10 m, the highest abundance was found to be during August (peak at 69.2% of total) and July (peak at 42.2% of total), respectively. The average abundance of each stage at different depths did fluctuate during the course of this study as seen in Figures 6, 7, 8, and 9.

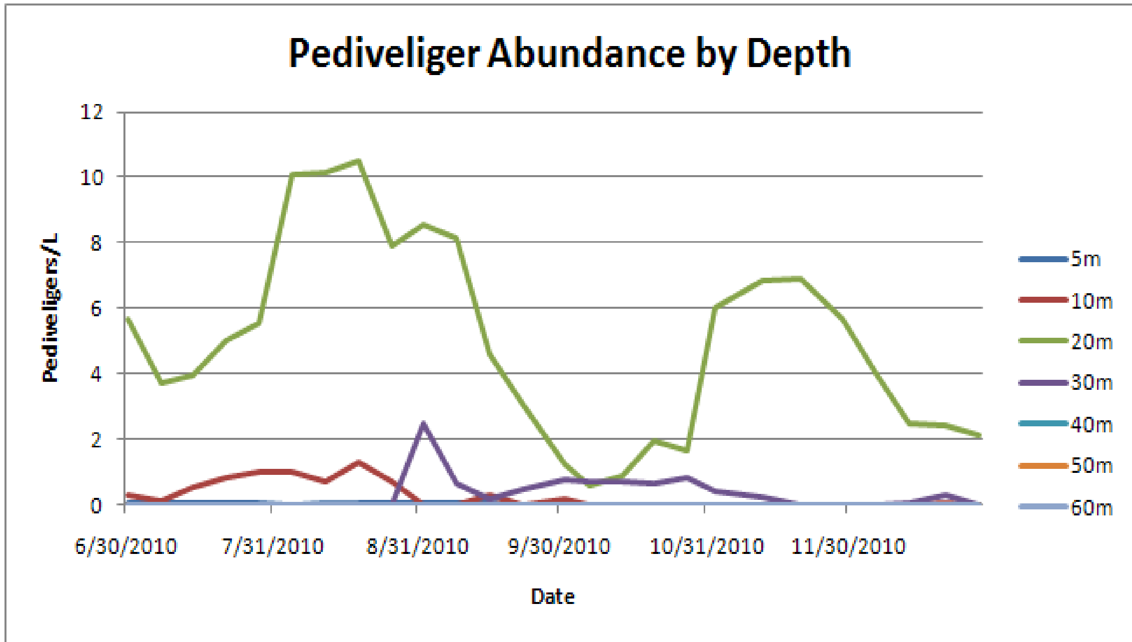


Figure 6. Average Pediveliger Abundance per L Calculated by Depth. Averages were taken weekly from 06/30/2010 to 12/28/2010.

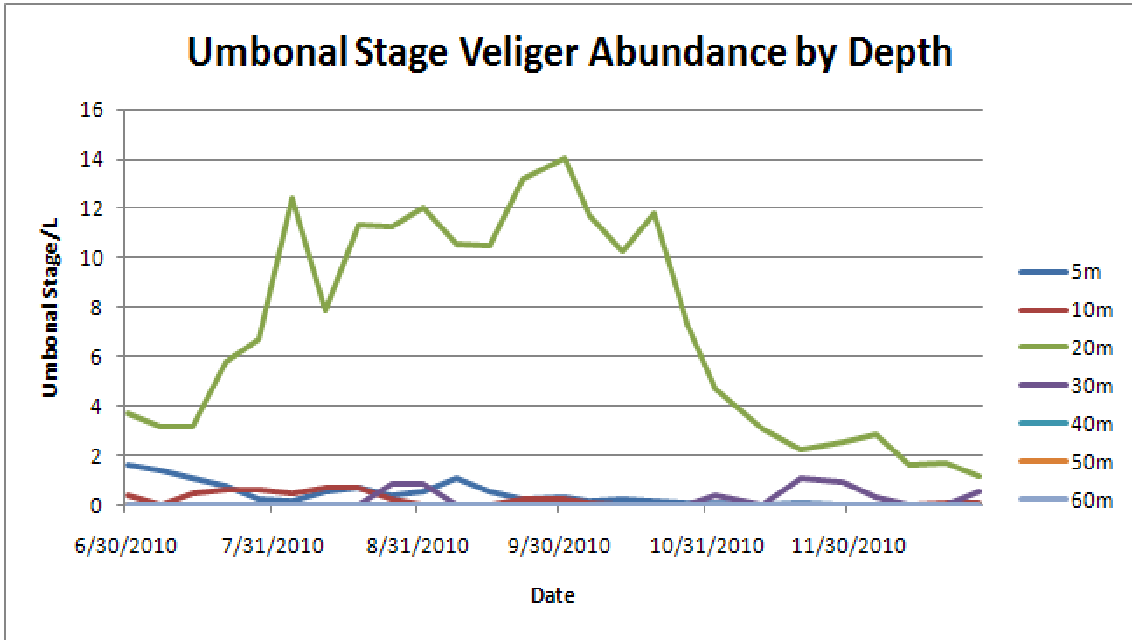


Figure 7. Average Umbonal Veliger Abundance per L Calculated by Depth. Averages were taken weekly from 06/30/2010 to 12/28/2010.

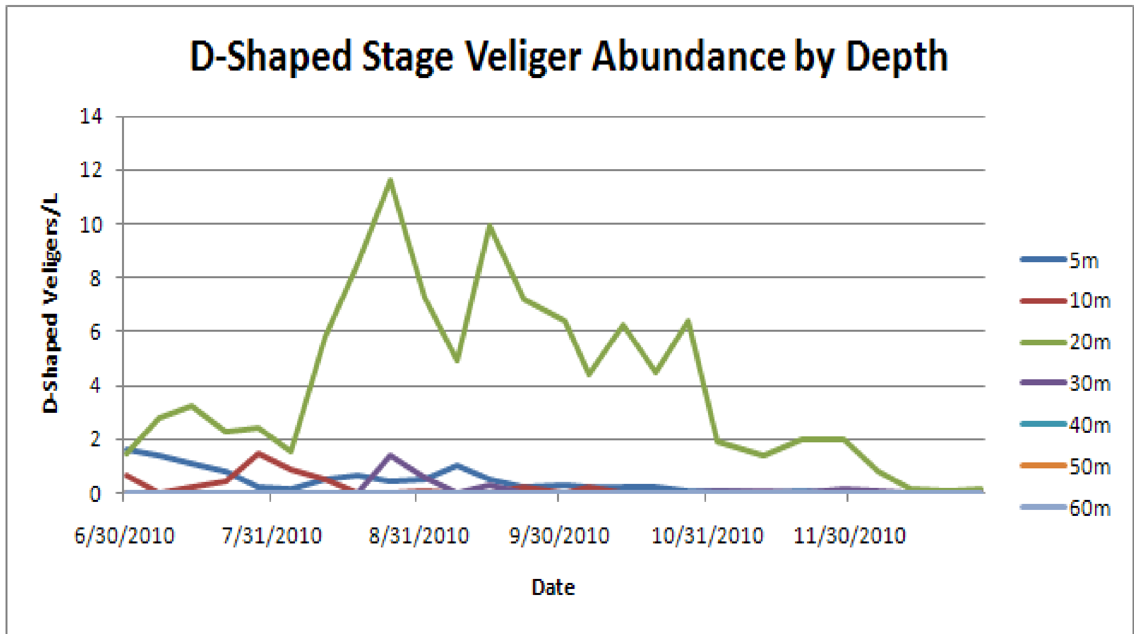


Figure 8. Average D-Shaped Veliger Abundance per L Calculated by Depth. Averages were taken weekly from 06/30/2010 to 12/28/2010.

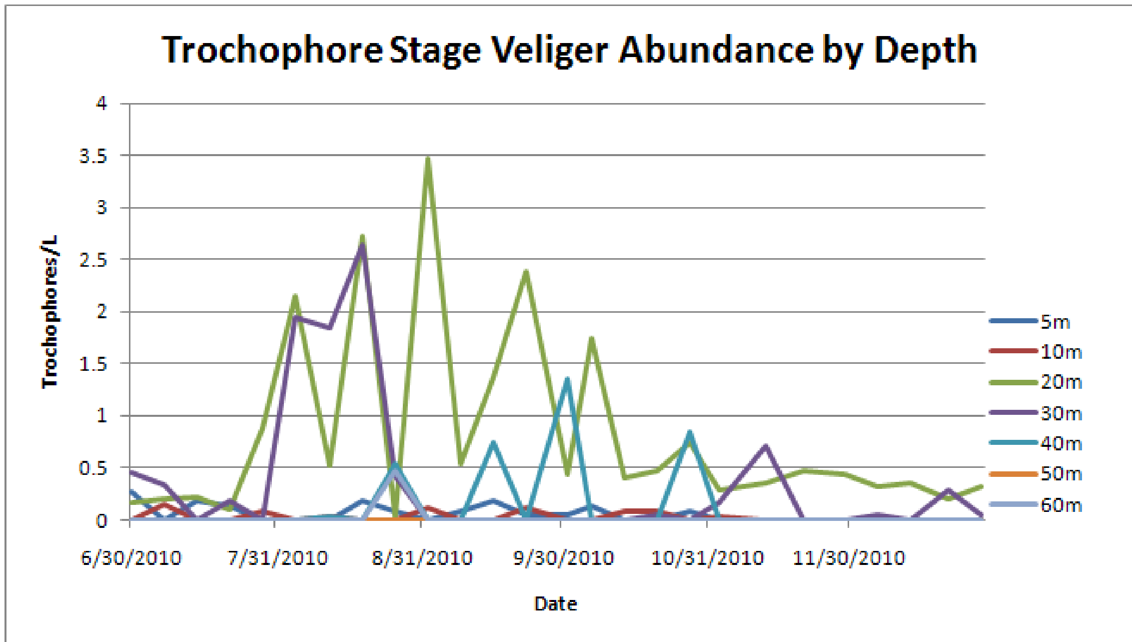


Figure 9. Average Trochophore Veliger Abundance per L Calculated by Depth. Averages were taken weekly from 06/30/2010 to 12/28/2010.

Results of Veliger Settlement at Specific Depths in Lake Mead

There was very little settlement present on the substrate slides at any of the specific depths from July 7, 2010 to December 28, 2010. There was a pronounced spike of settlers found on the slides retrieved on September 15, 2010 at depths of 5 m, 10 m, and 20 m.

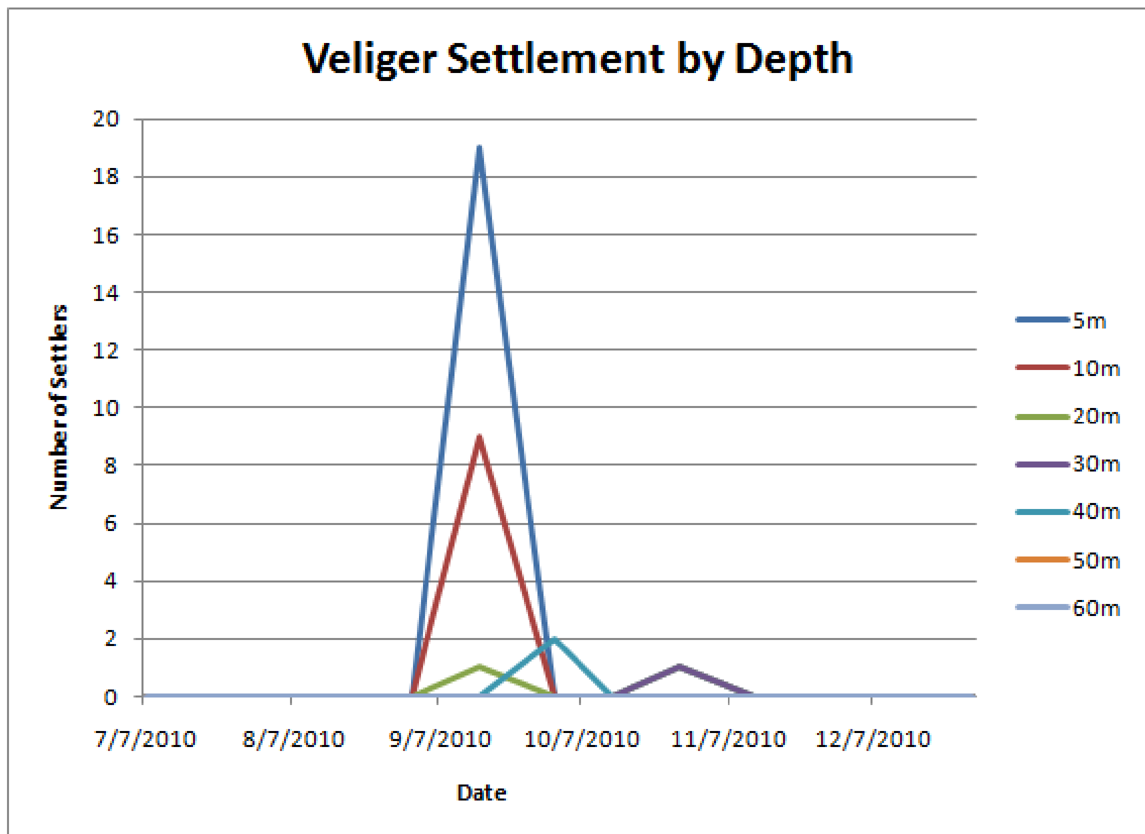


Figure 10. Biweekly Veliger Settlement at Specific Depths in Lake Mead from June 30, 2010 to December 28, 2010.

CHAPTER 5

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

Discussion of Results

Susceptibility of Quagga Mussels to Hot-Water Sprays

There has been an increasing concern among freshwater management agencies regarding the widespread recognition that the overland movement of boats and other watercraft are often responsible for spreading AIS (Johnson et al. 2001; Muirhead and McIssac 2005). Unfortunately, many of the efforts which have been directed towards the eradication and control of these environmental pests once they are already established in a freshwater body often result in additional ecological harm (Simberloff et al. 2005). Some agencies have concentrated on attempting to educate boaters about how individuals can reduce the likelihood of being a vector, but these prevention efforts have been rarer (Rothlisberger et al. 2010). The main concentration regarding slowing/preventing the spread of AIS to uncontaminated bodies of water has been on implementing inspection and decontamination procedures. Although inspection efforts can be very effective at catching a boat harboring an AIS before it enters the uncontaminated water body, procedures and management policies that also focus on decontamination procedures after leaving a contaminated water body will have an increased effectiveness (USBR 2010).

There have been many established and accepted methods of watercraft decontamination regarding preventing the spread of existing infestations of zebra and quagga mussels from already contaminated bodies of water (USBR 2010). But, certain methods which are eco-friendly, effective, and economical are more likely to be implemented than those which may eventually lead to further financial or ecological

problems (Piola 2009). One such method involves using thermal water at temperatures ≥60°C to decontaminate mussel-fouled surfaces, and has already been widely disseminated as a recommended boat washing procedure (Morse 2009). Some current protocol regarding thermal spray application has been based off findings in Morse's (2009) study, where it was found that spray temperatures ≥60°C are effective at killing 100% of the rinsed zebra mussels when applied for ≥10 s. Although, water temperatures of ≥60°C which are applied for <10 s may not be entirely effective at ensuring 100% zebra mussel mortality. The focus of the first study conducted for this thesis was to determine if quagga mussels were more or less susceptible to this method of decontamination than zebra mussels.

The present study found that at hot-water temperatures ≥60°C, a contact duration of 5 s was sufficient to induce 100% mortality in quagga mussels. For zebra mussels (Morse 2009), LT_{50} and LT_{99} at 1 s duration were both >80°C while they were 47.9°C and >80°C for quagga mussels in the present study (Table 5). At 5 s duration, LT_{50} and LT_{99} for zebra mussels were 54.6°C and 69.1°C while they were 47.2 and 58.8°C for quagga mussels. Accordingly, the results from this study suggest that quagga mussels are more susceptible to hot-water sprays than zebra mussels.

Just as all other bivalves, dreissenid mussels tend to close their shell valves tightly when mechanically disturbed. This offers protection for their soft tissues with a possible exception being the ventral byssal groove which is usually offered protection from the attachment surface. This valve closure prevents direct infiltration of the thermal spray to the soft tissues and slows the rate at which those tissues reach a lethal temperature. Therefore, it is assumed that heating of the soft tissues of the mussel to a lethal

temperature is a result of heat conduction across the shell valves. The difference in susceptibility of hot-water sprays between the two dreissenid mussels is probably a direct result of some important morphological differences between the two species. Quagga mussels have thinner shells (Zhulidov et al. 2006) and less tightly sealing shell valves (Claxton et al. 1997) than zebra mussels, which may allow the heating of the soft tissues of the quagga mussel to occur more rapidly than that of the zebra mussel. Another potential reason for this increased vulnerability may have to do with the impact of ambient temperature conditions and seasonal productivity variations on the acute thermal tolerance of dreissenid mussels (Elderkin and Klerks 2005). These factors may account for Morse's (2009) longer application time at 60°C for 100% kill in zebra mussels, as dreissenid mussels tend to have higher thermal elevated acute thermal tolerance temperature if they are acclimated in warmer waters within a laboratory setting before treatment (McMahon and Ussery 1995). The specimens of zebra mussels used in Morse's study (2009) were transferred from Hedges Lake (New York) and were acclimated to 20 ± 1°C water for two weeks prior to experimentation. The mussels in the present study, which experienced winter water conditions (i.e. lower temperature) within an actual field situation before treatment, and may have required higher temperatures or longer application times to achieve 100% mortality for different reasons than the laboratory acclimated mussels. Although the mussels were not acclimated to a constant higher water temperature as in the laboratory setting, the ambient conditions present in the field during the summer months such as increased stress from varying warmer water temperatures and direct sunlight may cause the mussels to be in a poorer physical condition than in the winter months where they would experience less thermal stress. If the mussels were taken

from Lake Mead during summer time when the surface water temperature ranges are higher (ranging from 25°C and 30°C), they may be more susceptible to the hot-water treatment (Robert F. McMahon, personal communication). It should be noted that in Morse's study the immediate mortality of the mussels after hot-water application was recorded, while it was found in the present study that some mussels did not die immediately after treatment (Figure 4). This means that there is a chance that the results may have been somewhat different had the two dreissenid species been tested by the same researchers under the same experimental conditions. The continuous mortality rates taken in this study verses the immediate mortality rates recorded in Morse's (2009) study could also be a potential reason that a longer application time was reported to make certain all zebra mussels were dead at 60°C. There was one conspicuous increase in mussel mortality present within the experiment that occurred between the 20°C group and the 40°C group (Figure 3) that can be explained by the fact that the upper thermal tolerance temperature for dreissenid mussels is reported to be around 30°C (McMahon and Ussery 1995; Karatayev et al. 1998).

Even so, the data obtained from this experiment mirrors the reported species-specific characteristic of the upper thermal limit of quagga mussels being lower than that of zebra mussels (Spidle et al. 1995; McMahon 1996; Mills et al. 1996). This vulnerability could be exploited by management agencies in regards to developing a more adaptable and efficient boat decontamination protocol which recreational boaters may be more apt to follow due to the less time needed to apply hot-water sprays ≥60°C to ensure 100% quagga mussel mortality whem compared to zebra mussels. There are many areas of boats and other various watercrafts which are capable of being subjected to direct

thermal spray (i.e. hull, trim tabs). These areas would only require hot-water application of ≥5 s at temperatures of ≥60°C, instead of the ≥10 s contact duration necessary to kill zebra mussels at the same temperature. Though this may not seem like a tremendous difference in application time, a vast majority of the boat area (i.e. hull, deck) would have the treatment time regarding boat decontamination reduced by half. This would appeal to both recreational boaters and government agencies because less money would be spent on the necessary time of labor required to conduct the entire decontamination procedure and it would allow boaters to leave the freshwater recreation area more quickly. The use of species-specific guidelines for boat decontamination procedures would be more agreeable to monitoring agencies in the western United States where water bodies are heavily infested specifically by quagga mussels (Benson 2010). In cases where the water body is infested by only zebra mussels or both zebra and quagga mussels could possibly be involved in fouling a boat, a duration of ≥10 s at temperature ≥60 ºC should be implemented. Freshwater regions with active surveillance of their specific dreissenid populations will be able to employ species-specific decontamination procedures most effectively as they can determine and use the hot-water decontamination standard most applicable toward their particular invasive mussel population.

Field Validation of Category I and Category II Watercraft Areas

Although the new information regarding the increased susceptibility of quagga mussels to thermal spray compared to zebra mussels will be quite useful in revising and developing watercraft decontamination standards and procedures where applicable, the direct application of this data can only be used to readily accessible areas of the watercraft capable of receiving the contact spray directly (Category I areas in Table 1).

Dreissenid mussels do display a tendency to settle in particularly well-sheltered areas of watercraft such as motors, anchors, intake and outlets, trim tabs, and centerboard slots (Morse 2009), where they may not be able to receive a direct hot-water spray and/or may come in contact with sprayed water as runoff from other surfaces where it may have cooled below the lethal temperatures. For these reasons, it was necessary to conduct experiments to evaluate the amount of time necessary for hot-water to be applied to these inaccessible areas (Category II in Table 1) in order to reach the most efficient and safe temperature resulting in 100% quagga mussel mortality. The inboard/outboard motor gimbal units of two separate boats were evaluated for this experiment, one in the summer and one in the winter. This was done because depending on the ambient temperature and conditions, the surface temperature of the gimbal units may vary; meaning the amount of time necessary to reach the lethal temperature in differing conditions may also vary. As expected, it took significantly longer than the Category I recommended duration of 5 s with 60°C hot-water for the top flush of the gimbal unit to reach the target lethal temperature at the bottom of the gimbal unit. The amount of time needed to achieve the target lethal temperature also varied with the specific season; a maximum of 43 s for the summer flush, and a maximum of 2 minutes and 7 s for the winter flush. This was probably due to the different surface area temperatures present between the two seasons.

In addition to areas which are inaccessible to hot-water sprays on watercraft, there are also areas which are not capable of withstanding temperatures in excess of 54°C. These areas may be made of materials that could be susceptible to heat-associated damage such as thick plastics or tubing. For these areas (Category III on Table 1), the determined 100% mortality rates for temperatures ≤54°C may be used to prevent such damage from

occurring. For the evaluation of Category III areas, the live and bait wells of the same recreational boat were tested in both summer and winter. The temperature of 54°C was used because it would require the least amount of additional contact duration to ensure 100% mussel mortality. As in the evaluation of the gimbal unit, the amount of time necessary to flush the live wells and bait wells was significantly longer than the necessary time regarding Category I areas.

In order for the information obtained from these experiments to be put to practical use in the real world, it was necessary to conduct field tests on actual boats encrusted with quagga mussels. The data regarding the most effective and least hazardous lethal temperature and duration regarding Category I areas could be applied directly to any area of the boat encrusted with mussels that could receive a direct spray. This was determined to be hot-water at a temperature of 60°C for a duration of 5 s. The same standard was used for both the winter and summer experiments because this spray would be directly contacting the mussels allowing the lethal temperature to heat the soft tissues of the mussels completely through heat conduction across the shell valves without interfering conduction from outside materials that may be protecting the mussels. For both winter and summer experiments, all Category I groups tested at this specific temperature and time combination had a resulting 100% quagga mussel mortality (Table 10, Table 12). The density of the experimental groups did vary between winter and summer, allowing many more smaller mussels to be killed per experimental group in the summer (mean $n = 105$) than the winter (mean $n = 47$). The average shell size between the summer and winter experimental group also varied at 6.57 ± 2.48 mm and 15.70 ± 4.27 mm, respectively. Although, the 100% mortality within the larger winter group offered

confirmation that this specific standard can be used to ensure 100% mortality among some of the hardiest of quagga mussels.

The validation experiments concerning the Category II areas, specifically the quagga encrusted gimbal units of the contaminated boats which were tested, were treated differently than the Category I areas because the hot-water spray could not directly contact the mussels colonized deep within the gimbal unit. For these experiments, a combination of the data obtained from the field test on emersed mussels and the evaluation of time needed to reach and sustain lethal temperatures in Category II areas was used.

For the summer validation, a hot-water flush (60°C) was applied to the top of the gimbal unit for a total of 48 s, 43 s required to heat the entire unit to the necessary lethal temperature and an additional 5 s to ensure 100% quagga mussel mortality. The results of the experiment showed that the application of hot-water to the gimbal unit for this amount of time did ensure 100% quagga mussel mortality in the two experimental groups. For the winter validation, the same technique was used on the gimbal unit regarding the flush, but the amount of time was increased in order to make sure the unit was heated to the necessary lethal temperature in the colder conditions. The hot-water flush (60°C) lasted for a duration of 2 minutes and 12 s, 2 minutes and 7 s to ensure the unit would be heated to the lethal temperature and 5 s to ensure 100% quagga mussel mortality. Of the two experimental groups, only one displayed 100% mortality while the other displayed 96% mortality. Since 100% mortality was not achieved in the second experimental group, this current combination of duration and lethal temperature should not be used for Category II areas in winter conditions. One aspect to examine why this specific

combination of duration and temperature did not work is in regards to the structure of the gimbal unit. A hot-water flush applied only to the top of the gimbal unit may not reach all of the settled quagga mussels within the sides of the hollow cylindrical structure. Therefore, a 2 min and 12 s rinse should have been conducted at both the top and the sides of the gimbal unit in order to make certain that all of the parts are heated to the necessary lethal temperature. Applying a hot-water was to only the top of the unit was shown not to be effective.

Veliger Collection and Substrate Colonization at Different Depths

Although adult quagga mussels cause the most obvious economic and ecological damage when introduced into an uncontaminated water body, i.e. clogging public facilities, producing odor problems, fouling other benthic organism, and affecting the ecosystem, the planktonic veliger stage is the most important and accessible to monitor (Wong et al. 2011). By monitoring the quagga mussel veliger populations and settlement rates, abundance and distribution data can be used to help understand how and when they may be most likely to impact a reservoir's biotic resources (e.g. fisheries, benthos, and planktonic community) and its cultural (e.g. water quality and water-delivery facilities) and recreational values (e.g. need for and cost associated with boat decontamination) (Wong et al. 2011). Information regarding the seasonal patterns of veliger abundance, abundance at specific depths, and competent pediveliger presence would indicate the most suitable time to treat facilities and implement preventive protocol to prevent biofouling from occurring.

The study conducted was part of a year-long monitoring program by the National Park Service to determine specific weekly veliger abundance and biweekly settlement

rate of quagga mussels at different depths in Lake Mead. Due to the fact that the study is ongoing, the six months data thus far collected from June 30, 2010 until December 28, 2010 was analyzed.

As found in a previous study (Mueting 2009), quagga mussel veligers were present within the water column of Lake Mead through the entire study period. It was found that a vast majority of the total veliger concentration was within a depth of 10 to 20 m, and most of the data analysis is in regards to the data obtained from the abundance of veligers from the 20 m tow. An analysis of the total veliger abundance revealed that there was a dramatic increase in the abundance (veligers/L) from the beginning of August 2010 where it peaked twice, once on August 18, 2010 (31 veligers/L for 20 m depth) and once on September 1, 2010 (30.5 veligers/L for 20 m depth). From this last peak, there was an immediate drop in abundance (22.8 veligers/L for 20 m depth) followed by a short increase and gradual decline until December 2010 (Figure 5). The reported optimum temperature for dreissenid larval development is 18°C (Sprung 1987) and in the metalimnion, the temperature from July to November typically ranges between 17°C and 21°C (Gerstenberger et al. unpublished data). The study confirmed this reporting a majority of the veligers found to be present between depths of 10 m to 30 m within the metalimnion. The dramatic increase in veligers during August and September 2010 is somewhat speculative because various factors can affect the abundance of *Dreissena* planktonic veligers, such as food quantity and quality, temperature, waves, hydrodynamics, and so on (Reid et al. 2010). Though, this phenomenon may just be potentially associated with water temperature and/or the natural reproduction cycle of adult quagga mussels in Lake Mead. The gradual decrease in veliger abundance from

September 2010 until December 2010 is probably due to a decrease in water temperature, but water temperature profiles should be used to determine if this is true as soon as they become available to confirm this suspicion.

In an analysis of the abundance data from the study period, it was found that there is a difference in the abundance of each quagga mussel veliger stage of quagga mussel at the different depths during the study period. The most important stage in regards to competency to settle is the pediveliger stage. During the study period it was found that a majority of pediveligers were present during the months of August and September 2010 (Figure 6), although there was also a noticeable peak of pediveliger abundance that occurred during the month of November (6.93 pediveligers/L) (Figure 6). There was also a marked decrease in pediveliger abundance during the month of October 2010 (Figure 6). During this month, there was a noticeable increase in the abundance of umbonal veligers (Figure 7), which is probably why there was not a pronounced decrease in the average total veliger abundance during that month (Figure 5). There were no reportable peaks in the analysis of the D-shaped veliger stage which seem to maintain a constant presence throughout the sampling period except for a slight decrease in the months of November and December (Figures 8). The trochophore veliger stage abundance seemed to follow no particular pattern and the amount of trochophores collected per sample varied week to week. They were found throughout the sampling period and seemed to decrease in abundance during the months of November and December (Figure 9).

An interesting aspect to note about the veliger sampling was that there was very little total veliger abundance (Figure 5) at the sampled depths of 5 m and 10 m, and there was very little to no veliger abundance at depths of 40 m, 50 m, and 60 m (Figure 5).

Although previous research has stated that the optimum temperature for larval development was in the deeper metalimnion (Sprung 1987), previous studies regarding quagga mussel settlement (Mueting 2009) have shown that a greater amount of mussel settlement on substrates placed at 10 m as compared to others placed at 20 m, 28 m, 37 m, 46 m, and 54 m. This could be the result of a variety of factors. One such factor could be that mussels tend to avoid direct sunlight. The mussels' avoidance of light could possibly be due to a natural instinct to avoid exposure to predators, avoid wave damage, or due to an increased water temperature caused by sunlight (Marsden and Lansky 2000). The shallower depths may be exposed to more light which causes the mussels to stay in deeper areas of water where they are protected from the light. Another such factor is temperature. Since the epilimnion is exposed to a variety of ambient factors such as air temperature and sunlight, the shallower depths may not be as conducive to veliger development as the deeper depths.

The other portion of the veliger monitoring study focused on substrate colonization by quagga mussels at specific depths. This was a biweekly sample started on June 30, 2010 until December 21, 2010 at specific depths of 5 m, 10 m, 20 m, 30 m, 40 m, 50 m, and 60 m. Unfortunately, during the course of this study little to no settlement took place on any of the slides at any of the depths. There was settlement on three separate occasions, September 15 (settlement at 5 m, 10 m, 20 m), October 1 (settlement at 40 m), and October 27, 2010 (settlement at 5 m, 20 m, and 30 m) (Figure 10). During these periods of time there were no noticeable changes in pediveliger abundance of which may have spurred or explained the random mussel settlement, although there was an increase in umbonal stage veligers during October around the time of the October settlement dates.

Though it is possible a variety of factors could have caused the lack of settlement during the experiment, the main cause is probably due to the limited period of time that each of the slides was kept submerged in the lake. Veligers typically do not settle, or settle at a reduced rate (10-20%) on substrates that have no microscopic biofilm (Marsden 1992; Kavuras and Maki 2003; Wainman et al. 1996). A biofilm is a gathering of glycoproteinacious film on a substrate which is then colonized by bacteria, diatoms, and protozoa (Wainman et al. 1996). Biofilms may increase the surface area of the substrate by providing more attachment sites or could possibly alter the surface chemistry to become more favorable to mussels (Kavouras and Maki 2003). It normally takes a biofilm up to two weeks to form on a substrate (Mueting 2010, Kavouras and Maki 2003). Since the substrate sampling occurred every two weeks, the plates were only given enough time to start accumulating a biofilm, therefore mussel settlement was unlikely to occur within the allotted sampling period.

Discussion of Research Questions

These two studies attempted to answer several research questions. The study regarding hot-water sprays as a means of watercraft decontamination for areas infested with quagga mussels was the focus of the majority of research questions. The first question that needed to be addressed was if the quagga mussel was more or less susceptible than the zebra mussel to hot-water spray. The first field study conducted found that quagga mussels are in fact more susceptible at hot-water spray and 100% mortality can be achieved when water temperatures ≥60°C are applied for 5 s while at the same temperature zebra mussels need the spray to be applied for a duration of 10 s (Table

4). The second question was in regards to determining the temperatures and exposure times needed to attain 100% mortality of adult quagga mussels following exposure to a hot-water spray. The field study found that the specific durations and temperatures necessary to achieve 100% mortality were ≥60°C water for 5 s, 54°C water for 10 s, 50°C water for 20 s, and 40°C for 40s (Table 3). The third research question was in regards to the duration of time necessary for hot-water to be applied to watercraft areas that are inaccessible to spray treatment (i.e. gimbal areas) in order to reach the most efficient and safe temperature for 100% quagga mussel mortality. This question was evaluated in the field where two standards were created, one for summer (48 s) and one for winter (2 min 12 s). Both of these times were evaluated on actual quagga encrusted watercraft gimbal units. The time standard for the summer application resulted in 100% mussel mortality for all experimental groups while the winter application time did not (Tables 13 and 15). Reasons for this discrepancy were discussed in a previous section. The last question regarding hot-water spray decontamination treatment had to deal with determining the amount of time needed for watercraft areas which cannot be flushed with ≥ 54°C/130°F water (i.e. ballast tanks and bladders) to reach the predetermined thresholds for 100% quagga mussel mortality. This data was obtained and recorded but was not evaluated in the field (Tables 8-11).

There were also two research questions which needed to be addressed in regards to the veliger collection and substrate colonization study. The first question was in regards to determining the specific depths at which most quagga mussels pediveligers are present between the months of June 2010 and December 2010. From the data obtained in this study, the most quagga mussel pediveligers are present within depth of 10-20 m

throughout the entire study period. It was also discovered that the total amount of veligers peaked from August 2010 to September 2010 and again from November to December 2010. The last research question was in regards to determining the specific depths at which quagga mussel settlement occurs the most during the months of June 2010 through December 2010. Unfortunately, there was a lack of settlement on a majority of the slides during this period and no substantial data could be made to answer this question.

Study Limitations

There were several study limitations with regards to all the evaluations presented within this thesis. In regards to the evaluations of time necessary to achieve hot-water temperatures lethal to quagga mussels in both Category II and Category III areas, one must take into account that these area can vary tremendously in size and materials. The times developed and recorded for these specific areas are applicable only to the specific watercraft equipment and specific areas tested and may not be correct for other areas. In these cases, it is necessary to use an infrared water temperature gauge to ensure that the surface material has, in fact, reached the lethal water temperature for the predetermined amount of time in order to make sure no viable quagga mussels are left after treatment. Another limitation involves the veliger abundance data within Lake Mead. All of the samples were taken from a specific location weekly (Sentinel Island, Lake Mead), and the data may not be applicable to other areas of the lake which may have more or less abundance. Lake Mead is a deep, complex ecosystem with complete stratification occurring about every other year that could also affect the veliger abundance. In addition to this, varying weather conditions, flow rate, water level, and additional factors could

have an impact on veliger abundance at any depth. Also veliger samples were not able to be taken every seven days consistently because of unforeseeable factors (i.e. illness, poor weather conditions, interagency scheduling conflicts). Samples were still taken as close to a week apart as possible, but the slight deviation between days in which the samples were collected could have had a minor effect on the veliger abundance and distribution data collected. The veliger abundance and colonization study is also only a portion of a year-long study being conducted, meaning that it is possible to see different peaks and different veliger stage abundances in the during the weeks that were not sampled (January-June).

Study Contributions

The data obtained from the study regarding the susceptibility of quagga mussels to hot-water sprays can be used by freshwater body management agencies as an additional resource to utilize when developing and revising watercraft decontamination standards, especially in the western United States where quagga mussels are the most prevalent dreissenid species (Benson 2010). The study also provides information about the decontamination of Category I, Category II, and Category III areas that may be useful in creating and modifying decontamination techniques for different watercrafts. Since hot-water is an eco-friendly, relatively cheap and easily accessible resource with low application time when compared to most other forms of watercraft decontamination, this study contributes to the notion that it is a great effective method of decontamination. As long as the procedures and standards are followed correctly, watercraft decontamination

by means of hot-water spray can be effective at preventing the spread of quagga mussels to uninfested freshwater bodies.

Contributions from the veliger abundance study include the potential development of a more efficient veliger sampling protocol from a new understanding of where the vast majority of veligers are present (10 m to 30 m). By sampling from 30 m in depth as opposed to 60 m in depth, one can accurately gauge the total amount of veligers present within the water column. Another inadvertent contribution concerning the study of substrate colonization of quagga mussels addresses information regarding lack of colonization present on the substrate slides between June and December. The lack of biofilm generated on the slides may be a reason for the low colonization during this period, and as most recreational boaters are not in the water for more than one day, the chance of colonization within a two week period may actually be quite low. Although it is still important to make sure boats are decontaminated in order to prevent the spread of quagga mussels, educating the boaters about the spread of quagga mussels and allowing simple desiccation procedures to take place before the individual enters another body of water may be both effective and cost efficient. This study also adds to the body of knowledge regarding veliger sampling using substrates. Because biweekly sampling is ineffective, it is suggested that substrates be left in the water for a period of three to four weeks in order to allow a biofilm to develop, increasing the probability of settlement.

Conclusions and Recommendations for Further Study

According to the data obtained from the studies testing the susceptibility of quagga mussels to hot-water spray as a means of watercraft decontamination, it is recommended

that hot-water sprays at 60°C for a duration of 5 s can be utilized to ensure 100% quagga mussel mortality under experimental and differing field conditions (winter and summer). If the water temperature is lower than this, 100% mortality cannot be achieved for that specific duration. It is recommended that a temperature of 60°C rather than a higher temperature because they are reported to have the same efficacy at the same durations, and higher temperatures may be hazardous to human health (Morse 2009). The 60°C/5 s standard is only to be used for readily accessible areas of the watercraft, and only used for mitigation of the quagga mussel. For other areas of watercraft (Category II and Category III), it is necessary to verify all surface areas are heated to the correct predetermined lethal temperature for the required amount of time to ensure 100% quagga mussel mortality. The results the study validating a time standard for a specific watercraft area (i.e. the gimbal unit) shows that developing a specific time standard may not be entirely effective for larger parts and under different weather conditions. Further research needs to be conducted regarding different areas on specific watercraft so that decontamination procedures can be developed depending on the type and model of boat contaminated with quagga mussels.

According to the data obtained from the studies monitoring veliger abundance and colonization rates of quagga mussels at different depths, there is a difference in abundance for each veliger stage of quagga mussel from June 30, 2010 to December 28, 2010. It was found that there is a low abundance of veligers at sampling depths of 5 m to 10 m, while a large amount of abundance was found between the depths of 10 m to 30 m. There were veligers present in lower concentrations at 40 m, 50 m, and 60 m, but the total count abundance data was higher proportionality for these regions because the sample

tow had to pass through depths of 20 m and 30 m, causing the net to collect veligers in the regions where there is the most reported abundance. This study shows that it may be more efficient to sample quagga mussels at a depth of 30 m rather than 60 m because the same relative proportion of veligers is present in both samples. This is because the largest abundance of veligers appears to occur from 10 m to 30 m from June 30, 2010 until December 28, 2010. This information may change with the months that were not analyzed in this study, but this new proposed protocol could work potentially work for the months analyzed. In the analysis of pediveliger presence within the lake, it was shown that a majority of the pediveligers are present between the months of August and September 2010, and November and December 2010. The highest percentage of veligers compared to the total collected sample was calculated to be during the months of November and December 2010. A majority of the pediveligers present were found at a depth between 10 m and 20 m. The lack of settlement on the substrates during the biweekly substrate sampling suggests that when monitoring substrates for veliger settlement, the substrate materials should be left out in the body of water for a period of greater than two weeks in order to ensure a biofilm develops and provides the mussels with an increased chance of settlement (Mueting 2009, Kavouras and Maki 2003). In order to ensure that effective and timely measures are employed to prevent quagga mussels from causing additional damage beyond human control continued monitoring of the dreissenid species within already infested freshwater bodies in addition to implementing practical methods of watercraft decontamination should be used to help prevent their spread to pure and uncontaminated water bodies.

APPENDIX 1

PROTOCOLS

BOR Veliger Sampling Protocol

The Lower Colorado Region Bureau of Reclamation Fisheries group is currently conducting monthly sampling for Quagga mussel veligers *Dreissena burgensis* on Lake Mohave. Water samples (one at each site) are being obtained near mid-channel at four sites on the lake including Willow Beach Marina, Placer Cove, Cottonwood Cove Marina, and Katherine Landing Marina. Samples are being collected following guidelines put forth by Kevin Kelly and Fred Nibling of the Bureau of Reclamation Technical Service Center in Denver, Colorado. Credit for this protocol should go to them as it is an adaptation of their original work. In addition to collecting water samples for analysis, water quality data is also being recorded. The following summarizes equipment needs as well as sampling, storage, and shipping methods.

Equipment

- 64 µm Plankton Tow Net (15 cm diameter opening)
- Water Quality probe (In-Situ Troll 9500 for recording date, time, Lat/Long, UTM and measuring temp, SpC, DO, pH, depth, turbidity, and TDS)
- 1 L spray bottle
- (4) Sample bottles (500 mL Nalgene HDPE bottles)
- Ethyl Alcohol (200 proof, preservative)
- Plastic electrical tape
- 1 gallon Ziploc bags
- Waterproof markers

- Data sheet on waterproof paper
- Ice chest with ice
- 2 gal. white vinegar (5% acetic acid, for plankton net decontamination)
- (2) 5 gal. buckets (one used as a decontamination container, one for WQ probe)
- Secchi disk (10.5 in.)
- 26 in. Aquavue scope (for use with the Secchi disk)

Sample Collection

In order to obtain the minimum sample volume of 1000 L for analysis, plankton nets are lowered and towed for a total of 60 meters. In actuality none of our four sites are 60m deep, so we instead use multiple tows at the same location until the plankton net has passed through 60 total meters. As an example, the max depth at the Katherine Landing site is 31-33m so we simply do two 30m tows to obtain our 60m sample. With the exception of Willow Beach Marina, all plankton net tows are vertical. At Willow Beach the current is too strong to allow for vertical tows so horizontal tows are taken. This is achieved by anchoring the boat, determining the flow rate (m/s), and holding the plankton net stationary below the surface for the appropriate duration.

After each tow a 1 L spray bottle is used to wash the net top to bottom from the outside to rinse veligers into the collection cup. The collection cup side screens are also washed top to bottom and then emptied into a 500mL Nalgene bottle. The collection cup is rinsed twice more with small amounts of water and emptied into the same 500mL bottle. Sample bottles are marked at the 375 mL line prior to each trip using a waterproof marker. This line is labeled level 1. By marking them before each trip we can ensure our samples are near the desire volume of 375 mL. The bottle is also labeled with the date,

location, and sample depth. Sample bottles are kept on ice while in the field and then refrigerated until they are shipped.

Once sampling at any site is complete the plankton net must be decontaminated before it can be used at the next site. The treatment recommended by Kelly and Nibling is to rinse the net with clean water to remove any remaining veligers and then completely immerse the net in white vinegar. We use two gallons of white vinegar in a five gallon bucket for decontamination. The plankton net is soaked for approximately 45 minutes between samples and the same vinegar bath is used following all samples. Plankton nets are thoroughly rinsed with clean water after each soaking and before collecting the next sample.

Water quality data is also being recorded at each sample site. Current parameters include temp (C°), depth (m), pH, SpC (μs/cm), DO (mg/L), turbidity (NTU), and TDS (mg/L). Secchi disk depth readings (with and without Aquavue scope) are also being taken at each site and are recorded in meters. For Secchi readings, the disk is lowered in the water until it is not visible by the naked eye and then it is slowly brought up to where it can be seen. This process is repeated in the same manner using the Aquavue scope. Other data taken at each site includes date, time, location name, air temp, wind speed/direction, and GPS coordinates (we are currently reporting data using both Lat/Long and UTM)

<u>Storage and Shipping</u>

Once sample bottles are back in our office they are taken out of the cooler and preserved using ethyl alcohol (200 proof). The ethyl alcohol is added until it is 25% of the final sample volume. After the alcohol has been added, the sample level on the bottle

is marked with a short line and labeled level after alcohol. Samples are refrigerated until they are ready to be shipped. For shipping, the sample bottle caps are screwed tight and the seam is taped closed using plastic electrical tape. Bottles are wrapped in disposable diapers and placed in Ziploc bags (this is done in case the bottles leak). Bottles are again put on ice and shipped in a cooler. Samples are analyzed by the Bureau of Reclamation office in Denver, CO.

Protocol courtesy of Jim Stolberg, Bureau of Reclamation, Boulder City, NV.

BOR Protocol for Analyzing Plankton Tows, Pumped Samples, and

Shallow Water Samples for *Dreissena* spp. Veliger Density

Scope and Application

This is a Reclamation method that was developed using the Standard Method 10200 G Zooplankton Counting Techniques, Standard Operating Procedure for Zooplankton Analysis and the US. Army Corps of Engineers (USACE) method for calculating *Dreissena* spp. veliger densities in water samples collected with a 63 µm plankton net.

Summary of Method

To avoid transporting live veligers in the sample, preserve each sample with 25% ethanol while in the field. Record the total volume of the sample (tow volume) and the volume of ethanol added to the concentrated sample. In the laboratory, the sample is added to an Imhoff settling cone with a venoset delivery system. The veligers are allowed to settle in the Imhoff cone for a minimum of 24 hours. Veligers are identified at the laboratory using cross-polarized light microscopy where they appear as a distinctive, bright "iron cross" among the other, darker planktonic material. Enumeration of veligers is performed with a Sedgwick-Rafter counting cell. The Sedgwick-Rafter counting cell chamber is divided lengthwise into three compartments and each compartment is counted separately, and then added together to determine the total number of veligers in 1mL of sample. Count five 1-mL aliquots from the same sample, record the number of veligers, and calculate the mean of the five counts. When the veliger concentration is very high, samples may be split with a Folsom plankton splitter or diluted with ultrapure deionized water (UPDI). It is possible to confuse veligers with Ostracods which also appear as a similar-shaped, bright "iron cross." However,

ostracods are kidney bean-shaped, and veligers are either round or D-shaped. Recount the cell to verify the veliger count.

Apparatus and Reagents

Dissecting microscope (10x-50x magnification) with cross polarized light filters

1-mL syringes or pipettes

Imhoff Cones set into a ringstand, with a venoset apparatus attached to the bottom

Sedgwick-Rafter counting cell (cover glass optional)

Small sieves with 45-µm mesh

50- and 500-mL beakers

15 mL Calibrated test tubes

UPDI

Isopropyl or ethyl alcohol

5% acetic acid solution

Analytical Procedure and Enumeration

1. All samples should be kept on ice or refrigerated from the time of collection. Record the total volume of the tow or the total volume of the watered filtered through the net into the sample cup (total volume sampled). Record the volume of ethanol that was added to preserve the sample or mark the levels on the sample bottle so that the discrete volumes can be recorded back in the laboratory.
2. Shake sample well and immediately pour into Imhoff cone with the venoset attachment. If the sample contains a large amount of debris, filter through a net as you pour the sample into the cone. Rinse the net contents thoroughly into cone with a wash bottle containing distilled water.

3. Allow to settle in the Imhoff cone for at least twenty four hours and up to 48 hours to allow veligers to settle.
4. Collect the first 15 mLs in a calibrated tube cover with parafilm and number it 1, collect the second 15 mls in a calibrated tube and cover with parafilm and number it 2. If there is still sediment remaining, continue collecting 15mLs at a time and number the tubes as they come off the cone. Note: the venoset may become clogged if the larger debris is not removed. If the smaller debris gets clogged, the flow is easily recovered by moving the clamp and squeezing the tube to move the constricting materials.

   Note: Generally it will not be necessary to examine the second 15 mLs under the microscope. However, the second collection may be used to verify that all of the veligers were collected in the first 15 mLs.
5. Pipette a 1-mL aliquot from a well-mixed sample and dispense into a Sedgwick-Rafter counting cell. If desired, a cover glass may be used.
6. Place the filled Sedgwick-Rafter cell under a dissecting microscope using cross polarized light. Examination of the counting cell is simplified by counting the cells by each compartment. Split or dilute the sample as needed to maintain a single layer of organisms, taking care to record dilutions or concentrations and factor them into the final count.
7. If needed, a drop of detergent in the Sedgwick-Rater cell will sink the microorganisms and reduce motion; however, veligers will sink fairly rapidly on their own.
8. Examine the contents of the cell and record the number of veligers present.

9. Repeat with same sample, using 1 mL aliquot for five counts, taking care to shake the sample container to keep the sample well mixed and the veligers suspended.
10. The mean of the five rafter cell counts is used to obtain the mean number of veligers per milliter in the sample.
11. The final concentration is then: $$\frac{C \times V'}{V'' \times V'''}$$

    Where C= average number of veligers counted per mL

    V′ is the volume of the concentrated sample (15 mLs)

    V″ is the volume counted (Since this is an average of 5 - 1mL counts, it is 1mL)

    V‴ is the volume of the total sample or plankton tow in L

QA/QC

If desired, the standard deviation may also be calculated to determine the frequency distribution and significant differences in the data. It is expected that the counts should not differ by greater than 10%, or all counts should be within 90% of the mean. If they do not, the reasons for the discrepancies should be evaluated and discussed in the data report.

To prevent cross contamination, all laboratory equipment and tools must be well cleaned. Utilizing a vinegar bath soak for a minimum of one hour to dissolve the veliger shells and prevent cross-contamination of samples. When possible Reclamation uses two sets of equipment, one for water bodies where zebra mussels have not been detected, and one for water bodies where zebra mussels have been detected.

Protocol courtesy of Denizse Hosler, Bureau of Reclamation, Denver, CO.

NALGENE™ Imhoff Settling Cone
Cat. No. 1000-0010

This settling cone is molded of transparent **polycarbonate (PC)** for excellent durability and clarity. The cone is shatterproof in normal handling and cleaning. It is fully graduated to allow convenient estimating at intervals. The leakproof polypropylene (PP) screw closure is easily removed for cleaning or withdrawal of sediment for gravimetric analysis.

To Clean

For accuracy, the cone must be clean.

1. Remove closure.
2. Wash cone and closure with a mild detergent.
3. Rinse thoroughly.

Do not use solvents, alkaline or abrasive cleansers or other abrasives such as steel wool or scouring powder. The cone and closure are autoclavable.

To Use*

1. Fill cone to the one-liter mark with well-mixed sample.
2. Place in an Imhoff cone rack (Cat. No. 1001-0010) and allow 45 minutes to settle.
3. Gently stir sides of cone with a rod or by spinning. Settle 15 minutes longer.
4. Record volume of settleable matter in the cone as mL/L.

Chemical Resistance

The cone is resistant to most aqueous solutions. **Do not use with strong acids, strong bases or organic solvents.**

* American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, 16th edition, APHA: Washington, D.C. 1985, section 209E ("Settleable Matter"), pp 98-99.

Related products available from Nalge

NALGENE Imhoff cone rack (Cat. No. 1001-0010)—Acrylic; holds up to three cones.
NALGENE settlometer kit (Cat. No. 1010-0507)—Includes polycarbonate jar, cover, centrifuge tubes, polypropylene paddle and tube closures, instructional booklet and data sheet.

For further information, see the current NALGENE Labware Catalog or contact Technical Service, Nalge Company, a Subsidiary of Sybron Corporation, Box 20365, Rochester, New York 14602-0365 U.S.A. Telephone: (716) 586-8800 (ext.660) Fax: (716) 586-3294.

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Data Sheet for Quagga Mussels Veliger Enumeration

QUAGGA MUSSEL VELIGER COLLECTION AND BENCH SHEET

Entity: ____________________

Location: ____________________ Submission ID: ____________________

Date Started: ____________________ Date Finished: ____________________

Time Started: ____________________ Time Finished: ____________________

Collected by: ____________________ Collected by: ____________________

Meter Reading Start: ____________________ Meter Reading End: ____________________

PHYSICAL PARAMETERS:

Temperature: ____________ °C Conductivity: ____________ µS/cm

Dissolved Oxygen: ____________ mg/L pH: ____________ Units

Chlorine Free: ____________ mg/L Chlorine Total: ____________ mg/L

Turbidity: ____________ NTU

ENUMERATION: Workgroup ID:

Total Volume Collected: ____________ Gallons Total Volume Collected: ____________ Liters

Total Concentrate Volume: ____________ Sub-sample Volume: ____________

Ethanol Volume Used: ____________ Raw Water Concentrate Volume: ____________

Volume Filtered: ____________ Final Concentrate Volume: ____________

Sample: ____________ Counted By: ____________ Date & Time: ____________

Slide #	1	2	3	4	5	6	7	8	9	10	Mean #
Track 1:											
Track 2:											
Track 3:											
Track 4:											
Total:											

*Veliger Count per Liter: ____________

Sample: ____________ Counted By: ____________ Date & Time: ____________

Slide #	1	2	3	4	5	6	7	8	9	10	Mean #
Track 1:											
Track 2:											
Track 3:											
Track 4:											
Total:											

*Veliger Count per Liter: ____________

Analyst Signature: ____________ Date: ____________

Supervisor Signature: ____________ Date: ____________

* Formula for Veliger Count Per Liter:
total mean # of veligers x (total concentrate volume/raw water concentrate volume) x (volume filtered/final concentrate volume) x raw water concentrate volume = total # of veligers/total volume collected in liters = veliger count per liter

APPENDIX 2

DATA TABLES

Adjusted Total Veliger Abundance (veligers/L) by Depth and Date

	5m	10m	20m	30m	40m	50m	60m
6/30/2010	3.73	1.07	9.77	0	0	0	0
7/7/2010	3.88	0	8.91	0	0	0	0
7/14/2010	2.78	1.09	9.33	0	0	0	0
7/21/2010	2.32	1.74	11.49	0	0	0	0
7/28/2010	0.95	3.14	13.93	0	0	0	0
8/4/2010	0.59	2.39	24.73	0	0	0	0
8/11/2010	1.37	2.02	22.76	0	0	0	0
8/18/2010	1.91	1.67	31.01	0	0	0	0
8/25/2010	1.46	0.73	29.28	0.48	0	0	0
9/1/2010	1.87	0	30.54	1.35	0	0	0
9/8/2010	2.82	0	22.82	0	0	0	0
9/15/2010	1.77	0	25.34	0	0	0	0
9/22/2010	0.55	0.45	25.69	0	0	0	0
10/1/2010	0.50	0.30	21.87	0	0	0	0
10/6/2010	0.59	0.21	18.44	0	0	0	0
10/13/2010	0.46	0.06	17.82	0	0	0	0
10/20/2010	0.36	0.07	18.74	0	0	0	0
10/27/2010	0.32	0	16.16	0	0	0	0
11/2/2010	0.14	0.023	12.92	1.03	0	0	0
11/12/2010	0	0	11.70	0.03	0	0	0
11/20/2010	0.045	0	11.62	0.10	0	0	0
11/29/2010	0	0	10.63	0	0	0	0
12/6/2010	0	0	8.04	0.19	0	0	0
12/13/2010	0	0	4.62	0	0	0	0
12/21/2010	0.09	0.07	4.29	0.25	0	0	0
12/28/2010	0.05	0.03	3.74	0.47	0	0	0

Adjusted Pediveliger Abundance (veligers/L) by Depth and Date

	5m	10m	20m	30m	40m	50m	60m
6/30/2010	0.04	0.32	5.65	0	0	0	0
7/7/2010	0.04	0.09	3.71	0	0	0	0
7/14/2010	0.03	0.51	3.93	0	0	0	0
7/21/2010	0.04	0.80	5.02	0	0	0	0
7/28/2010	0.03	1.00	5.53	0	0	0	0
8/4/2010	0.02	1.02	10.06	0	0	0	0
8/11/2010	0.04	0.73	10.12	0	0	0	0
8/18/2010	0.04	1.29	10.52	0	0	0	0
8/25/2010	0.04	0.69	7.90	0	0	0	0
9/1/2010	0.06	0	8.52	2.50	0	0	0
9/8/2010	0.07	0	8.14	0.63	0	0	0
9/15/2010	0.04	0.30	4.60	0.17	0	0	0
9/22/2010	0.01	0	3.04	0.49	0	0	0
10/1/2010	<0.01	0.15	1.23	0.75	0	0	0
10/6/2010	<0.01	0	0.59	0.72	0	0	0
10/13/2010	0	0	0.86	0.72	0	0	0
10/20/2010	0	0	1.95	0.65	0	0	0
10/27/2010	0	0	1.67	0.82	0	0	0
11/2/2010	0	0	6.01	0.41	0	0	0
11/12/2010	0	0	6.85	0.21	0	0	0
11/20/2010	0	0	6.93	0	0	0	0
11/29/2010	0	0	5.65	0	0	0	0
12/6/2010	0	0	4.02	0	0	0	0
12/13/2010	0	0	2.47	0.08	0	0	0
12/21/2010	0	0.08	2.39	0.27	0	0	0
12/28/2010	<0.01	0	2.14	0	0	0	0

Adjusted Umbonal Veliger Abundance (veligers/L) by Depth and Date

	5m	10m	20m	30m	40m	50m	60m
6/30/2010	1.59	0.39	3.73	0	0	0	0
7/7/2010	1.37	0	3.15	0	0	0	0
7/14/2010	1.09	0.47	3.17	0	0	0	0
7/21/2010	0.77	0.61	5.79	0	0	0	0
7/28/2010	0.23	0.61	6.75	0	0	0	0
8/4/2010	0.14	0.46	12.40	0	0	0	0
8/11/2010	0.50	0.71	7.83	0	0	0	0
8/18/2010	0.68	0.66	11.37	0	0	0	0
8/25/2010	0.41	0.22	11.25	0.82	0	0	0
9/1/2010	0.50	0	12.00	0.83	0	0	0
9/8/2010	1.05	0	10.54	0	0	0	0
9/15/2010	0.50	0	10.52	0	0	0	0
9/22/2010	0.23	0.21	13.19	0	0	0	0
10/1/2010	0.32	0.19	14.05	0	0	0	0
10/6/2010	0.18	0.10	11.76	0	0	0	0
10/13/2010	0.23	0.01	10.28	0	0	0	0
10/20/2010	0.18	0.02	11.84	0	0	0	0
10/27/2010	0.09	0.03	7.35	0	0	0	0
11/2/2010	0.05	0	4.73	0.41	0	0	0
11/12/2010	0	0	3.11	0	0	0	0
11/20/2010	0.05	0	2.22	1.11	0	0	0
11/29/2010	0	0	2.55	0.90	0	0	0
12/6/2010	0	0	2.87	0.27	0	0	0
12/13/2010	0	0	1.63	0	0	0	0
12/21/2010	0	0.08	1.71	0	0	0	0
12/28/2010	0	0.04	1.15	0.57	0	0	0

Adjusted D-Shaped Veliger Abundance (veligers/L) by Depth and Date

	5m	10m	20m	30m	40m	50m	60m
6/30/2010	1.59	0.65	1.49	0	0	0	0
7/7/2010	1.37	0	2.80	0	0	0	0
7/14/2010	1.09	0.25	3.20	0	0	0	0
7/21/2010	0.77	0.42	2.29	0	0	0	0
7/28/2010	0.23	1.46	2.41	0	0	0	0
8/4/2010	0.14	0.91	1.55	0	0	0	0
8/11/2010	0.50	0.50	5.83	0	0	0	0
8/18/2010	0.68	0	8.55	0	0	0	0
8/25/2010	0.41	0	11.61	1.37	0	0	0
9/1/2010	0.50	0.10	7.27	0.58	0	0	0
9/8/2010	1.046	0	4.95	0	0	0	0
9/15/2010	0.50	0	9.92	0.26	0	0	0
9/22/2010	0.23	0.19	7.24	0	0	0	0
10/1/2010	0.32	0	6.36	0	0	0	0
10/6/2010	0.18	0.22	4.44	0	0	0	0
10/13/2010	0.23	0	6.26	0	0	0	0
10/20/2010	0.18	0	4.48	0	0	0	0
10/27/2010	0.09	0.023	6.39	0	0	0	0
11/2/2010	0.05	0	1.90	0.04	0	0	0
11/12/2010	0	0	1.39	0.04	0	0	0
11/20/2010	0.05	0	1.99	0	0	0	0
11/29/2010	0	0	1.99	0.13	0	0	0
12/6/2010	0	0	0.84	0.07	0	0	0
12/13/2010	0	0	0.16	0	0	0	0
12/21/2010	0	0	0.07	0	0	0	0
12/28/2010	0	0	0.16	0	0	0	0

Adjusted Trochophore Veliger Abundance (veligers/L) by Depth and Date

	5m	10m	20m	30m	40m	50m	60m
6/30/2010	0.27	0	0.16	0.46	0	0	0
7/7/2010	0	0.16	0.20	0.33	0	0	0
7/14/2010	0.18	0	0.22	0	0	0	0
7/21/2010	0.14	0	0.10	0.18	0	0	0
7/28/2010	0	0.08	0.88	0	0	0	0
8/4/2010	0	0	2.15	1.94	0	0	0
8/11/2010	0	0.04	0.52	1.84	0.02	0	0
8/18/2010	0.18	0	2.72	2.64	0	0	0
8/25/2010	0.09	0	0.03	0.43	0.55	0	0.47
9/1/2010	0	0.12	3.47	0	0	0	0
9/8/2010	0.09	0	0.55	0	0	0	0
9/15/2010	0.18	0	1.37	0	0.74	0	0
9/22/2010	0.05	0.11	2.38	0	0	0	0
10/1/2010	0.05	0	0.43	0	1.36	0	0
10/6/2010	0.14	0	1.73	0	0	0	0
10/13/2010	0	0.08	0.40	0	0	0	0
10/20/2010	0	0.08	0.48	0.05	0	0	0
10/27/2010	0.09	0	0.75	0	0.85	0	0
11/2/2010	0	0.04	0.29	0.17	0	0	0
11/12/2010	0	0	0.36	0.70	0	0	0
11/20/2010	0	0	0.48	0	0	0	0
11/29/2010	0	0	0.44	0	0	0	0
12/6/2010	0	0	0.32	0.05	0	0	0
12/13/2010	0	0	0.36	0	0	0	0
12/21/2010	0	0	0.20	0.29	0	0	0
12/28/2010	0	0	0.32	0.05	0	0	0

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