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Decontamination protocols for watercraft and wildland firefighting equipment in preventing the spread of invasive quagga (Dreissena rostriformis bugensis) and zebra (Dreissena polymorpha) mussels

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DECONTAMINATION PROTOCOLS FOR WATERCRAFT AND WILDLAND FIREFIGHTING EQUIPMENT IN PREVENTING THE SPREAD OF INVASIVE QUAGGA (*DREISSENA ROSTRIFORMIS BUGENSIS*) AND ZEBRA (*DREISSENA POLYMORPHA*) MUSSELS

By

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A dissertation submitted in partial fulfillment
of the requirements for the

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December 2014

ABSTRACT

Decontamination protocols for watercraft and wildland firefighting equipment in preventing the spread of invasive quagga (*Dreissena rostriformis bugensis*) and zebra (*Dreissena polymorpha*) mussels

by

Ashlie Watters

Dr. Shawn Gerstenberger, Examination Committee Chair
Dean, School of Community Health Sciences
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Quagga and zebra mussels (*Dreissena rostriformis bugensis* and *Dreissena polymorpha*) are two invasive species introduced via ballast water discharged by large oceanic cargo ships to the North American Great Lakes in the late 1980s. Once established, the mussels spread quickly. In January 2007, *D. rostriformis bugensis* was discovered in Lake Mead, NV-AZ, and in that same year, mussels were confirmed further south on the Colorado River in Lakes Mojave and Havasu. Dreissenids clog water intake pipes, water filtration systems, and electric generating plants. The mussels also ruin boat motors, damage recreational equipment, and once established in the reservoir, routine maintenance is necessary to avoid further damage. Prevention is the most cost effective and environmentally protective tool against the further spread of dreissenids. Preventive measures include decontaminating vessels and gear that could transport the mussels, thus restricting the transport and subsequent release of these potentially harmful species. Decontaminating methods examined in this dissertation include high pressure, hot-water sprays and chemical applications. The aim of this research is to evaluate three techniques for preventing the further spread of dreissenids: 1) high pressure water sprays to remove

dreissenids from watercraft, 2) hot-water spray to kill *D. polymorpha*, and 3) use of quaternary ammonium compounds, Quat™ 128 and Quat™ 256 for decontaminating wildland firefighting equipment. Using 3000 psi of water to remove dreissenids from watercraft is accomplished at a faster rate when the vessel has been out of the water for at least one week in the summer and two weeks in the winter compared to being fresh out of the water (week 0). *D. polymorpha* 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 54°C for 10 s were shown to be 100% lethal. The effectiveness of Quat™ 128 and Quat™ 256 on killing adult dreissenids was examined over time at four concentrations: 0, 1%, 3%, and 5%. The results of the study show that all treatment groups of Quat™ 256 are 100% lethal to adult dreissenids within 36 h. Dreissenid veligers were also examined over time at different concentrations of Quat™ 128 and Quat™ 256: 0.25%, 0.5%, and 0.75%; 0.1%, 0.25%, and 0.5%, respectively, at different water temperatures: 2, 16, and 30°C, and at different ambient temperatures: 2, 15, 30, and 43°C. Given all the factors of chemical toxicity, water temperature, and ambient temperature, 40 min exposure time to 0.25% Quat™ 128 or 0.1% Quat™ 256 induced 100% mortality in dreissenid veligers. This project will provide baseline data that will be used to draft standard and effective decontamination protocols for watercraft and wildland firefighting equipment exposed to dreissenids throughout the country and in particular in the western U.S.

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I would not be at this point in my life, writing a dissertation and soon to be a PhD graduate, without the endless support of my mentors, family, and friends. To me, this has seemed like a never ending journey and I am so grateful to have all of you with me to the end. Here's to you.

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TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGMENTS v

LIST OF TABLES x

LIST OF FIGURES xii

LIST OF ACRONYMS & ABBREVIATIONS xiv

CHAPTER 1 INTRODUCTION 1
Purpose of the Study 3
Research Questions 5
Hypotheses 6

CHAPTER 2 REVIEW OF RELATED LITERATURE 11
Aquatic Invasive Species 11
Aquatic Invasive Species Background 11
Quagga and Zebra Mussel Background 14
Spread and Distribution of Dreissenids 14
Morphological Differences 19
Physiological Tolerances 21
Life Cycle and Reproduction 23
Settlement 26
Vectors of Spread 29
Control Methods 36

CHAPTER 3 METHODOLOGY 44
Pressurized Water Spray to Remove Dreissenids on Watercraft 44
Research Objectives 44
Methods 45
Quagga Mussels 45
Zebra Mussels 49
Statistical Analysis 50
Susceptibility of Zebra Mussels to Hot-water Spray 51
Research Objectives 51
Methods 51
Specimen Collection and Experimental Design 51
Hot-water Spray Watercraft Validation 54
Statistical Analysis 55
The Efficacy of Quaternary Ammonium Compounds on Killing Dreissenids 55
Research Objectives 55
Methods 56
Specimen Collection 56

Lethal Effects of Quat™ 128 and Quat™ 256 on Dreissenids 57
Effects of Water Temperature on the Efficacy of Quat™ 128 and Quat™ 256 60
Effects of Ambient Temperature on the Efficacy of Quat™ 128 and Quat™ 256 63
Statistical Analysis 65

CHAPTER 4 FINDINGS OF THE STUDY 66
Analysis of Data 66
Pressurized Water Spray to Remove Dreissenids from Watercraft 66
Susceptibility of Zebra Mussels to Hot-water Spray 71
Hot-water Spray Watercraft Validation 73
The Efficacy of Quaternary Ammonium Compounds on Killing Dreissenids 75
Lethal Effects of Quat™128 and Quat™ 256 on Dreissenids 75
Effects of Water Temperature on the Efficacy of Quat™ 128 and Quat™ 256 81
Effects of Ambient Temperature on the Efficacy of Quat™ 128 and Quat™ 256 85
Quat™ 128 85
Quat™ 256 92

CHAPTER 5 DISCUSSION, CONCLUSIONS, & RECOMMENDATIONS 94
Discussion of Results 94
Pressurized Water Spray to Remove Dreissenids on Watercraft 94
Susceptibility of Zebra Mussels to Hot-water Spray 97
The Efficacy of Quaternary Ammonium Compounds on Killing Dreissenids 101
Discussion of Research Questions 107
Study Limitations 110
Study Contributions 111
Recommendations for Further Study 113
Conclusions 114

APPENDIX A KANSAS DEPARTMENT OF WILDLIFE, PARKS AND TOURISM COLLECTION PERMIT 116

APPENDIX B NATIONAL PARK SERVICE COLLECTION PERMIT 117

APPENDIX C VELIGER COLLECTION PROCEDURES 119

APPENDIX D HOT-WATER SPRAY TREATMENT FIGURES 123

APPENDIX E STANDARDIZED PROTOCOL 127

BIBLIOGRAPHY 130

VITA 143

LIST OF TABLES

Table 1 Experimental design for pressurized water spray to remove *D. rostriformis bugensis* and *D. polymorpha* on watercraft.....47
Table 2 Zebra mussels tested per treatment group.....52
Table 3 Experimental design for testing lethal effects of Quat™ 128 and Quat™ 256 on dreissenid adults.....58
Table 4 Experimental design for testing lethal effects of Quat™ 128 on dreissenid veligers.....59
Table 5 Experimental design for testing lethal effects of Quat™ 256 on dreissenid veligers.....60
Table 6 Experimental design for testing the effects of water temperature on duration of exposure to Quat™ 128 and Quat™ 256 on killing dreissenid veligers.....61
Table 7 Effects of ambient temperature on mortality of veligers.....64
Table 8 Final working solutions of Quat™ 128 and Quat™ 256 with added veliger sample.....64
Table 9 Time to remove high or low densities of *D. rostriformis bugensis* and *D. polymorpha* from watercraft in summer and winter seasons using 1500 or 3000 psi of water on week 0.....69
Table 10 Time to remove high or low densities of *D. rostriformis bugensis* from watercraft in the winter seasons using 1500 or 3000 psi of water on week 2.....69
Table 11 Time to remove high or low densities of *D. rostriformis bugensis* from watercraft in the winter seasons using 1500 or 3000 psi of water on week 4.....70
Table 12 Time to remove high or low densities of *D. rostriformis bugensis* and *D. polymorpha* from watercraft in the summer season using 1500 or 3000 psi of water on week 1.....70
Table 13 Mortality rate of *D. polymorpha* under different treatments by day 72
Table 14 Estimated LD_{50} and LD_{99} values and their 95% confidence limit for hot water spray treatments on *Dreissena polymorpha* at 1 s, 2 s, 5 s, and 10 s application durations.....72
Table 15 Mortality rates of *D. rostriformis bugensis* exposed to Quat™ 128 after 48 h.....78
Table 16 Mortality rates of *D. rostriformis bugensis* exposed to Quat™ 256 after 48 h.....78
Table 17 Mortality rates of *D. polymorpha* exposed to Quat™ 128 after 48 h.....79
Table 18 Mortality rates of *D. polymorpha* exposed to Quat™ 256 after 48 h.....79
Table 19 Minimum time to induce 100% mortality in *D. rostriformis bugensis* using 0.25, 0.5, and 0.75% Quat™ 128 in 2, 16, 30, and 43°C ambient temperatures.....87

Table 20 Minimum time to induce 100% mortality in *D. rostriformis bugensis* using 0.1, 0.25, and 0.5% Quat™ 256 in 2, 16, 30, and 43°C ambient temperatures.....93

LIST OF FIGURES

Figure 1 Examples of AIS threats to Lake Mead, NV-AZ.....12
Figure 2 Native ranges of *D. rostriformis bugensis* and *D. polymorpha* 15
Figure 3 *D. rostriformis bugensis* and *D. polymorpha* distribution in the U.S. June 2014.....17
Figure 4 *D. rostriformis bugensis* and *D. polymorpha* distribution in the U.S. March 2007.....18
Figure 5 Morphological differences between *D. rostriformis bugensis* and *D. polymorpha*.....20
Figure 6 Life cycle of dreissenids from the free-living larval phase to the sessile adult phase.....25
Figure 7 *D. rostriformis bugensis* attached with byssal threads to the hull of a watercraft.....27
Figure 8 Common areas where *D. rostriformis bugensis* and *D. polymorpha* adults and veligers are found on watercraft.....30
Figure 9 Vessel out of the water for several weeks showing water leaking from engine.....33
Figure 10 Examples of firefighting equipment: A) backpack sprayer, B) water tender, C) portable tank, D) portable pump, E) helicopter bucket, and F) fire engine.....35
Figure 11 Watercraft heavily encrusted with *D. rostriformis bugensis*.....46
Figure 12 Group of mussels segmented for high pressure testing.....48
Figure 13 LANDA pressure washer used in the high pressure experiments.....48
Figure 14 Boat lift used for the summer season, Wilson Lake high pressure experiments.....50
Figure 15 PolyScience programmable heated circulator water bath.....53
Figure 16 Dreissenids being exposed to varying water temperatures.....62
Figure 17 Encrusted pontoon boat from Wilson Lake, Kansas.....74
Figure 18 Cumulative mortality of adult *D. rostriformis bugensis* exposed to three concentrations (0, 1, 3, and 5%) of (a) Quat™ 128 and (b) Quat™ 256.....76
Figure 19 Cumulative mortality of adult *D. polymorpha* exposed to three concentrations (0, 1, 3, and 5%) of (a) Quat™ 128 and (b) Quat™ 256.....77
Figure 20 Cumulative mortality of (a) *D. rostriformis bugensis* and (b) *D. polymorpha* veligers exposed to three concentrations (0, 0.25, 0.5, and 0.75%) of Quat™ 128.....80
Figure 21 Cumulative mortality of (a) *D. rostriformis bugensis* and (b) *D. polymorpha* veligers exposed to three concentrations (0, 0.1, 0.25, and 0.5%) of Quat™ 256.....80
Figure 22 (a) *D. rostriformis bugensis* and (b) *D. polymorpha* exposed to water temperatures of 2, 16, and 30°C (no chemical) for 1, 5, 10, 20, and 40 min.....82
Figure 23 *D. rostriformis bugensis* mortality rate (%) after 1 min exposure to (a) Quat™ 128 and (b) Quat™ 256.....83

Figure 24 *D. polymorpha* mortality rates after 1 min exposure to (a) Quat™ 128 and (b) Quat™ 256.....84
Figure 25 *D. polymorpha* mortality rates after 5 min exposure to (a) Quat™ 128 and (b) Quat™ 256.....85
Figure 26 Mortality rate for *D. rostriformis bugensis* exposed to three different Quat™ 128 concentrations that have been stored for (a) 1, (b) 5, and (c) 10 days at 2°C and three different Quat™ 256 concentrations that have been stored for (d) 1, (e) 5, and (f) 10 days at 2°C.....88
Figure 27 Mortality rate (%) for *D. rostriformis bugensis* exposed to three different Quat™ 128 concentrations that have been stored for (a) 1, (b) 5, and (c) 10 days at 16°C and three different Quat™ 256 concentrations that have been stored for (d) 1, (e) 5, and (f) 10 days at 16°C.....89
Figure 28 Mortality rate (%) for *D. rostriformis bugensis* exposed to three different Quat™ 128 concentrations that have been stored for (a) 1, (b) 5, and (c) 10 days at 30°C and three different Quat™ 256 concentrations that have been stored for (d) 1, (e) 5, and (f) 10 days at 30°C.....90
Figure 29 Mortality rate (%) for *D. rostriformis bugensis* exposed to three different Quat™ 128 concentrations that have been stored for (a) 1, (b) 5, and (c) 10 days at 43°C and three different Quat™ 256 concentrations that have been stored for (d) 1, (e) 5, and (f) 10 days at 43°C.....91

LIST OF ACRONYMS & ABBREVIATIONS

ADBAC	Alkyl Dimethyl Benzyl Ammonium Chlorides
AIS	Aquatic Invasive Species
ANSTF	Aquatic Nuisance Species Task Force
CPL	Cross Polarized Microscopy
DDAC	Diakyl Dimethyl Ammonium Chloride
DNA	Deoxyribonucleic acid
min	minute
PCR	Polymerase Chain Reaction
psi	pound per square inch
Quat	Quaternary Ammonium Compound
QZAP	Quagga Zebra Mussel Action Plan
s	seconds
SNK	Student–Newman–Keuls
THM	Trihalomethane
U.S.	United States
U.S. EPA	United States Environmental Protection Agency
U.S. FWS	United States Fish and Wildlife Service
U.S.FS	United States Forest Service
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

Lakes Mead and Mohave, located in Lake Mead National Recreation Area, are responsible for the successful development of the Southwestern U.S. Not only does the National Recreation Area provide aquatic habitat for a wide variety of wildlife and caters to eight million visitors per year (of those, most recreate on the water), but the reservoirs deliver drinking water and hydropower for electricity for more than 25 million people in California, Nevada, and Arizona (Rosen, Turner, Goodbred, & Miller, 2012). Lake Mead is the largest reservoir by volume (3.5 x 10 m^3) in the U.S. with four inflows, and three basins, plus variable seasonal and annual operational patterns (LaBounty & Burns, 2005).

Lakes Mead and Mohave provide favorable environmental conditions for the quagga mussel (*Dreissena rostriformis bugensis*), an invasive species, such as warm water, high calcium concentrations, hard substrates, suitable pH, and sufficient dissolved oxygen (Cross et al., 2011). In January 2007, *D. rostriformis bugensis* was discovered in Boulder Basin of Lake Mead (LaBounty & Roefer, 2007). This was the first known occurrence of an established dreissenid population in the western U.S. and the first known North American quagga mussel infestation of a large water body which was not previously infested by another dreissenid species, the zebra mussel (*Dreissena polymorpha*) (Wong, Gerstenberger, Baldwin, & Moore, 2012). While the quagga mussel was discovered in 2007, based on growth analysis studies, they are believed to have been introduced in 2003 or 2004 (Wong, Gerstenberger, Baldwin, & Moore, 2012; McMahon, 2011).

The economic impact of *D. rostriformis bugensis* and *D. polymorpha* in North America has been estimated at $1 billion/year (U.S. Army Corps of Engineers, 2002). In 2008, the National Park Service spent $5 million to create an inspection and decontamination program, U.S. Fish and Wildlife Services spent $1.8 million for its aquatic invasive species (AIS) program, and the U.S. Geological Survey spent $0.2 million for dreissenid support (Turner et al., 2011). The mussels clog water intake pipes, water filtration systems, and electric generating plants. The mussels also ruin boat motors, damage recreational equipment, and once established in the reservoir, routine maintenance is necessary to avoid further damage. For example, to maintain operations and prevent further damage and spread of *D. rostriformis bugensis* in the Colorado River Aqueduct, the Metropolitan Water District of Southern California plans to spend between $10-15 million per year (Wong & Gerstenberger, 2011).

D. rostriformis bugensis alter the ecosystem by increasing water clarity and bioaccumulating contaminants. With their efficient filtering capabilities, *D. rostriformis bugensis* remove suspended materials and nutrients from the water, making little or none available for native aquatic species that feed on the same nutrients (Claudi & Mackie, 1994). Both *D. rostriformis bugensis* and *D. polymorpha* are responsible for shifting the food web from a pelagic-based to a benthic-based one in Lake Erie which can create a new pathway for contaminant transfer to top predators (Hogan, Marschall, Folt, & Stein, 2007). This same effect may occur in Lake Mead, and monitoring protocols are in place to assess the issue. After *D. rostriformis bugensis* became established in Lake Mead, water clarity increased by 13% and chlorophyll concentrations declined by 45% (Wong et al., 2013). Mercury levels have been measured in *D. rostriformis bugensis* in Lake

Mead, and the baseline research shows potential in using *D. rostriformis bugensis* as a biomonitor of overall lake health because of their ease in collection, sedentary lifestyle, and wide distribution (Mueting & Gerstenberger, 2010).

As of 2014, there are no water bodies in the Pacific Northwest region (including Wyoming and Alaska) of the U.S. that have tested positive for *D. rostriformis bugensis* or *D. polymorpha*. If an introduction and successful establishment were to happen, it could result in control and management costs amounting to tens of millions of dollars annually with potentially larger ecological damage costs. The region has invested billions of dollars to maintain and increase native fish, primarily salmon and steelhead trout, and this investment could be at risk (Independent Economic Analysis Board, 2013). Prevention is the most cost effective and environmentally protective tool to stop the further spread of *D. rostriformis bugensis* and *D. polymorpha*. Preventive measures include decontaminating boats and gear that could transport the mussels, and restricting the transport and subsequent release of these potentially harmful species. Decontaminating techniques include high pressure sprays, hot water sprays, mandatory desiccation times, and chemical applications.

Purpose of the Study

The objective of this research is to fulfill action items put forth by the "Quagga and Zebra Mussel Action Plan for Western Waters" (QZAP) prepared by the Western Regional Panel on Aquatic Nuisance Species as recommendations for the Aquatic Nuisance Species Task Force (ANSTF). The QZAP identifies top priority actions to prevent spread and control of existing *D. rostriformis bugensis* and *D. polymorpha*

populations. Specific prevention strategies include 1) implementation of mandatory inspection and decontamination practices at infested waters, 2) continued development of effective watercraft and equipment inspection and decontamination protocols and standards, 3) adoption of protocols and standards in Western states, 4) establishment and implementation of strong, consistent law enforcement programs, and 5) development of a standardized model and strategy for risk assessment for water bodies (QZAP, 2010). The Western Regional Panel on Aquatic Nuisance Species was formed in 1997 to help limit the introduction, spread, and impacts of aquatic nuisance species into the Western Region of North America. This panel of public and private entities was formed by a provision in the National Invasive Species Act of 1996 (U.S. Fish and Wildlife Services, 2014). Because of high fecundity, planktonic veliger stage, and ability to attach to substrates with byssal threads (Ram & McMahon, 1996), dreissenids have easily and quickly spread to other lakes and reservoirs in the Upper and Lower Colorado River and have the potential to spread further. Several strategies have been employed to mitigate and control their spread. To fulfill action items from the QZAP, the aim of this research is to evaluate three methods of dreissenid decontamination to reduce their further spread: 1) high pressure, hot water spray for use on watercraft, 2) perform field validation tests confirming that hot-water spray kills *D. polymorpha*, and 3) the use of 8.45% of didecyl dimethyl and n-alkyl dimethyl benzyl ammonium chlorides found in Quat™ 128 and 16.9% of didecyl dimethyl and n-alkyl dimethyl benzyl ammonium chlorides found in Quat™ 256 for wildland firefighting equipment.

Research Questions

1. Is 1500 or 3000 psi of water more effective for removing 100% *D. rostriformis bugensis* and *D. polymorpha* (alive or dead) from a watercraft using high pressure spray in winter and summer seasons?
2. What is the minimum amount of time required to remove 100% *D. rostriformis bugensis* and *D. polymorpha* (alive or dead), using pressurized water spray, from an encrusted watercraft in winter and summer seasons?
3. If a watercraft has been stored out of the water for more than one day, will it be easier to remove attached *D. rostriformis bugensis* and *D. polymorpha* compared to being fresh out of the water?
4. Is *D. rostriformis bugensis* more or less susceptible than *D. polymorpha* to hot-water spray?
5. What are the temperatures and exposure times needed to attain 100% mortality of *D. polymorpha* following exposure to a hot-water spray?
6. Will 100% mortality of *D. polymorpha* be reached at a spray temperature of 60°C for 5 s?
7. Are Quat™ 128 and Quat™ 256 effective in killing adult and veliger *D. rostriformis bugensis* and *D. polymorpha*?
8. What is the lowest concentration of Quat™ 128 and Quat™ 256 that is effective in killing 100% of adult and veliger *D. rostriformis bugensis* and *D. polymorpha*?
9. Is *D. rostriformis bugensis* more or less susceptible than *D. polymorpha* to Quat™ 128 and Quat™ 256 treatment?

10. Will water temperature have an effect on the potency of Quat™ 128 and Quat™ 256 in killing adult and veliger *D. rostriformis bugensis* and *D. polymorpha*?
11. Will the strength of Quat™ 128 and Quat™ 256 be affected by differing ambient temperatures?

Hypotheses

$H_0$1: 3000 psi of water will not be more effective in achieving 100% removal rate of *D. rostriformis bugensis* and *D. polymorpha* on watercraft when compared to 1500 psi of water

H_A1: 3000 psi of water will be more effective in achieving 100% removal rate of *D. rostriformis bugensis* and *D. polymorpha* on watercraft when compared to 1500 psi of water

The Recommended Uniform Minimum Protocols and Standards for Watercraft Interception Programs for Dreissenid Mussels in the Western United States suggests to use a power wash unit capable of spraying at least 4 gallons/min with a nozzle pressure of 3,000 psi of water or greater (not to exceed 3,500 psi) to remove attached visible mussels from all exposed surfaces of the watercraft (Zook & Phillips, 2012). However, if a lower pressure (1500 psi of water) with shorter time of use is effective in removing 100% of dreissenid mussels, the time and cost can be minimized.

$H_0$2: *D. rostriformis bugensis* and *D. polymorpha* will not be removed from watercraft at a faster rate, using 1500 and 3000 psi of water, in the summer season compared to the winter season

H_A2: *D. rostriformis bugensis* and *D. polymorpha* will be removed from watercraft at a faster rate, using 1500 and 3000 psi of water, in the summer season compared to the winter season

With warmer temperatures in the summer season, the byssal threads of dreissenids dry out and decompose at a faster rate compared to the cooler temperatures of the winter season. The strength of the byssal threads will be reduced and it will take less time to remove them using high pressure water spray.

$H_0$3: It will not be faster to remove *D. rostriformis bugensis* and *D. polymorpha* from a watercraft using high pressure water spray that has been out of the water for 30, 14, and 7 days compared to day 0 when the watercraft is fresh out of the water

H_A3: It will be faster to remove *D. rostriformis bugensis* and *D. polymorpha* from a watercraft using high pressure water spray that has been out of the water for 30, 14, and 7 days compared to day 0 when the watercraft is fresh out of the water

When a watercraft is pulled from the water on day zero, the mussels are presumed alive and the byssal thread attachment is the strongest. The longer the watercraft sits out of the water, byssal threads begin to dry out and lose attachment strength, making it easier to remove the mussels with high pressure water spray. Adult mussels will be used

as a test target to establish standards in watercraft decontamination. In the quagga and zebra mussel life cycle, individuals are most resilient during the adult stage (Claudi & Mackie, 1994; Watters, Gerstenberger, & Wong, 2013). If 100% removal rate is reached for all adults, juveniles and veligers would be eliminated as well.

$H_0$4: *D. rostriformis bugensis* will not be more susceptible to hot-water spray than *D. polymorpha*

H_A4: *D. rostriformis bugensis* will be more susceptible to hot-water spray than *D. polymorpha*

The upper thermal limit of *D. rostriformis bugensis* is lower than that of *D. polymorpha* (Mills et al., 1996). *D. rostriformis bugensis* are reported to have thinner shells than *D. polymorpha*, meaning they may be more susceptible to hot-water sprays than *D. polymorpha* (Zhulidov et al., 2006).

$H_0$5: As the temperature and duration of the hot-water spray increases, the susceptibility of *D. polymorpha* mortality will not increase

H_A5: As the temperature and duration of the hot-water spray increases, the susceptibility of *D. polymorpha* mortality will increase

A recent study using *D. rostriformis bugensis* found 100% were killed at ≥ 5 seconds with temperatures ≥ 60°C, while 100% mortality rates were reached at ≥ 10, 20, and 40 seconds at 54°C, 50°C, and 40°C), respectively (Comeau et al., 2011).

$H_0$6: *D. rostriformis bugensis* and *D. polymorpha* veligers will not be more susceptible to Quat™ 128 and Quat™ 256 compounds compared to adults

H_A6: *D. rostriformis bugensis* and *D. polymorpha* veligers will be more susceptible to Quat™ 128 and Quat™ 256 compounds compared to adults

D. rostriformis bugensis and *D. polymorpha* veligers are more vulnerable to chemical applications compared to adults because they are significantly smaller and they lack a protective shell to close when the chemical is sensed (Claudi & Mackie, 1994; Watters, Gerstenberger, & Wong, 2013).

$H_0$7: Quat™ 128 and Quat™ 256 will not be more effective in killing *D. rostriformis bugensis* and *D. polymorpha* veligers in warmer water

H_A7: Quat 128 and Quat 256 will be more effective in killing *D. rostriformis bugensis* and *D. polymorpha* veligers in warmer water

Both species of dreissenids are generally intolerant of elevated temperatures beyond 30°C (Cohen, 2008). *D. rostriformis bugensis* show rapid mortality at 30°C and *D. polymorpha* will die at 36°C (McMahon 1996, Spidle et al. 1995). Increased water temperature can potentially kill dreissenid mussels even with lower chemical solution strength as they may be more stressed.

$H_0$8: The effectiveness of Quat™ 128 and Quat™ 256 in killing *D. rostriformis bugensis* and *D. polymorpha* veligers will not be reduced when exposed to warmer ambient temperature

H_A8: The effectiveness of Quat™ 128 and Quat™ 256 in killing *D. rostriformis bugensis* and *D. polymorpha* veligers will be reduced when exposed to warmer ambient temperature

The toxicity of ammonium compound is not only associated with concentration and duration of exposure, but is also temperature dependent (Martin, Mackie, & Baker, 1993). Apart from water temperature, after the solution is exposed to the ambient hot temperature in the field situation, such as summer time in the arid Southwest, the concentration and strength of these two compounds may be reduced as volatilization may occur at higher ambient temperature.

CHAPTER 2

REVIEW OF RELATED LITERATURE

Aquatic Invasive Species

Aquatic Invasive Species Background

Aquatic invasive species (AIS) (also referred to as nonindigenous or non-native) are aquatic organisms, including plants, animals, or microbes, that are introduced and become established in another ecosystem beyond their natural range (U.S. Fish and Wildlife Services, 2013). Their presence can have significant ecological and economic consequences harming the native ecosystems and can negatively affect commercial, agricultural, or recreational activities dependent on those ecosystems. The rate of introduction of AIS into new areas has accelerated because of increases in population, international trade, and travel (U.S. Fish and Wildlife Services, 2013). Typically, AIS are inadvertently introduced to new environments through humans, animals, and equipment which came into contact with contaminated water.

Some AIS, other than *D. rostriformis bugensis* and *D. polymorpha*, that are a threat to the Southwest region in the U.S., particularly Lake Mead, include didymo (*Didymosphenia geminata*), Eurasian watermilfoil (*Myriophyllum spicatum*), and silver carp (*Hypophthalmichthys molitrix*) (Figure 1). *D. geminata is* a free-living microscopic stalked diatom which forms unusually high quantities of stalk material, smothering the plants and animal species living in the reservoir (Kilroy & Unwin, 2011). *D. geminata* gives the appearance of used toilet paper, making the area where it bloomed unsightly for anglers and people recreating. *M. spicatum* is easily spread by fragments transported on boats and boating equipment, such as the trailer and prop. This aquatic plant has the

potential to reduce water quality, smother native plant species, and provide habitat for other invasive species (Eiswerth, Donaldson, & Johnson, 2000). *H. molitrix* has the potential to cause enormous damage to native fish species because it feeds on plankton required by native larval fish (Nonindigenous Aquatic Species Database, 2011). This carp species could also have a negative impact on recreational boating, as the sound and vibration of boat motors startle the fish, causing them to frantically jump out of the water, damaging boats, equipment, and injuring people.

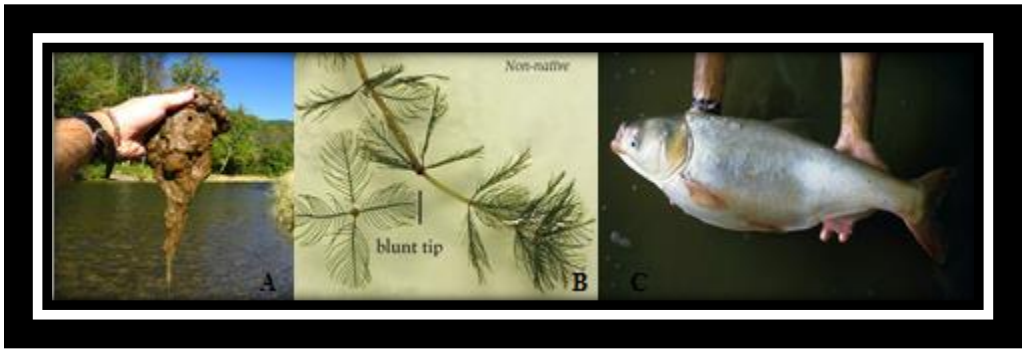


Figure 1. Examples of AIS threats to Lake Mead, NV-AZ. **A)** *Didymosphenia geminata* **B)** *Myriophyllum spicatum* **C)** *Hypophthalmichthys molitrix* Images from **A)** Pennsylvania Fish and Boat Commission, **B)** Lake George Association, and **C)** USGS, Nonindigenous Species Database. Retrieved [05 Apr 2014] from **A)** http://www.fish.state.pa.us/water/habitat/ans/didymo/faq_didymo.htm, **B)** http://www.lakegeorgeassociation.org/what-we-do/Invasive-Species/Eurasian-Watermilfoil.asp, and **C)** http://nas.er.usgs.gov/queries/factsheet.aspx?speciesID=549

Impacts of invasive species are second only to habitat destruction as a cause of global biodiversity loss (Lawler et al., 2006). AIS are one of the largest threats to the ecosystems and economies of the U.S. Approximately 49% of the species on the threatened or endangered species lists are at risk primarily because of predation or

competition with exotic species (Wilcove, Rothstein, Dubow, Phillips, & Losos, 1998). AIS can harm native and endangered species through predation, competition, introduction of parasites or diseases, habitat changes, and water quality impacts. AIS can also alter ecosystem processes and functions, including energy, nutrient and contaminant flows, sedimentation and erosion rates, and evapotranspiration rates (ANSTF, 2012).

New introductions of AIS cause widespread economic damages to fisheries, maritime infrastructure, recreational venues and equipment, water supply systems, and other resources and infrastructure. The U.S. invests more than $120 billion per year in damage and control costs to combat all invasive species (Pimentel, Zuniga, & Morrison, 2005). For instance, between 1989 and 1995, $69 million was spent across a range of industries that maintained infrastructure susceptible to mussel fouling on operations and maintenance issues (O'Neill, 1997). As the world trade network continues to grow, and climate continues to change, the number and frequency of introduced aquatic species are expected to increase.

AIS can have a negative effect on human health. While more research is needed to evaluate if there is a link between the transports of microorganisms to outbreaks of public disease, there is a direct link to human injury resulting from AIS. People can be severely injured if hit by *H. molitrix* jumping out of the water or cut from the sharp edges of *D. rostriformis bugensis* or *D. polymorpha* mussel shells found in recreation areas.

Quagga mussels (*D. rostriformis bugensis*), Asian clams (*Corbicula fluminea*), and New Zealand mudsnails (*Potamopygus antipodarum*) are three major aquatic invasive species found in Lakes Mead and Mohave. *C. fluminea* and *P. antipodarum* have not been as destructive as the quagga mussel, as they are not biofoulers nor do they

filter large amounts of lake water, thus removing plankton from the water column, and they are not as prolific as *D. rostriformis bugensis*.

Quagga and Zebra Mussel Background

Spread and Distribution of Dreissenids

D. polymorpha were first discovered by the Russian naturalist, Peter Pallas, in the backwaters of the Ural River, near the Caspian Sea, in 1769 (Figure 2) (Ludyanskiy, McDonald, & MacNeil, 1993). After canals were built, international trade increased, and extensive shipping between major European ports allowed *D. polymorpha* to spread quickly from the native region to other countries in Europe during the first half of the 19th century (Karatayev et al., 2007). *D. rostriformis bugensis* were first discovered and described by Nicolai Ivanovich Andrusov, a Russian geologist, in 1897. He named the species after the "quagga", an extinct African relative to the zebra animal (Mills, Rosenberg, Spidle, Ludyankiy, Pligin, & May, 1996; May & Marsden, 1992). *D. rostriformis bugensis* are native to a coastal lake near the Black Sea called the Dnieper-Bug (Figure 2). Although international ship traffic went through this area, *D. rostriformis bugensis* did not spread into Western Europe like *D. polymorpha* and it was not until the 1980s that *D. rostriformis bugensis* were found in the Don River system of Russia (Karatayev et al., 2007).

Figure 2. Native ranges of *D. rostriformis bugensis* and *D. polymorpha* (Son, 2007)

Both species of *Dreissena* were introduced into the North American Great Lakes via ballast water discharged by large oceanic cargo ships in the late 1980s (Carlton, 2008), which led to a successful establishment (Holeck et al., 2004). The zebra mussel was first documented in North America in 1988 in Lake St. Clair (Hebert, Muncaster, & Mackie, 1989). However, there is evidence that *D. polymorpha* was detected in Lake Erie on natural gas wellheads and well markers in 1986. Less than a year later, *D. polymorpha* was found off the shore of a water treatment plant and fouling vessels on Lake Erie. As the population increased in 1988, it is hypothesized that the *D. polymorpha* then spread to Lake St. Clair (Carlton, 2008). Once established, they spread quickly to all the Great Lakes and then entered eight river systems such as the St. Lawrence, Hudson, Mississippi, Ohio, Illinois, Tennessee, Susquehanna, and Arkansas (Ludyanskiy,

McDonald, & MacNeil, 1993) (Figure 3). Again, *D. polymorpha* spread at a faster rate than *D. rostriformis bugensis* by colonizing two times as many states as *D. rostriformis bugensis* and over 15 times more water bodies by 2008 (Karatayev et al., 2011). The first occurrence of the quagga mussel in North America was documented in 1989 in Lake Erie (Mills, Dermott, Roseman, Dustin, Conn, & Spidle, 1993). This morphologically and genetically different species of *Dreissena* was identified as a different species and then given the name quagga mussel in 1991 (May & Marsden, 1992). Once established in the Midwest and eastern parts of the U.S., *D. rostriformis bugensis* eventually moved towards the southwest region, crossing the 100th Meridian (Figure 4). *D. rostriformis bugensis* were discovered in Boulder Basin of Lake Mead, Nevada on January 7, 2007, and that same year, mussels were confirmed further south on the Lower Colorado River in Lake Mohave, Nevada-Arizona and Lake Havasu, Arizona (LaBounty & Roefer, 2007). The quagga mussel establishment in Lake Mead is the first major invasion of *Dreissena* in western North America. This is still an early invasion when comparing it to the invasion to the Great Lakes or Western Europe.

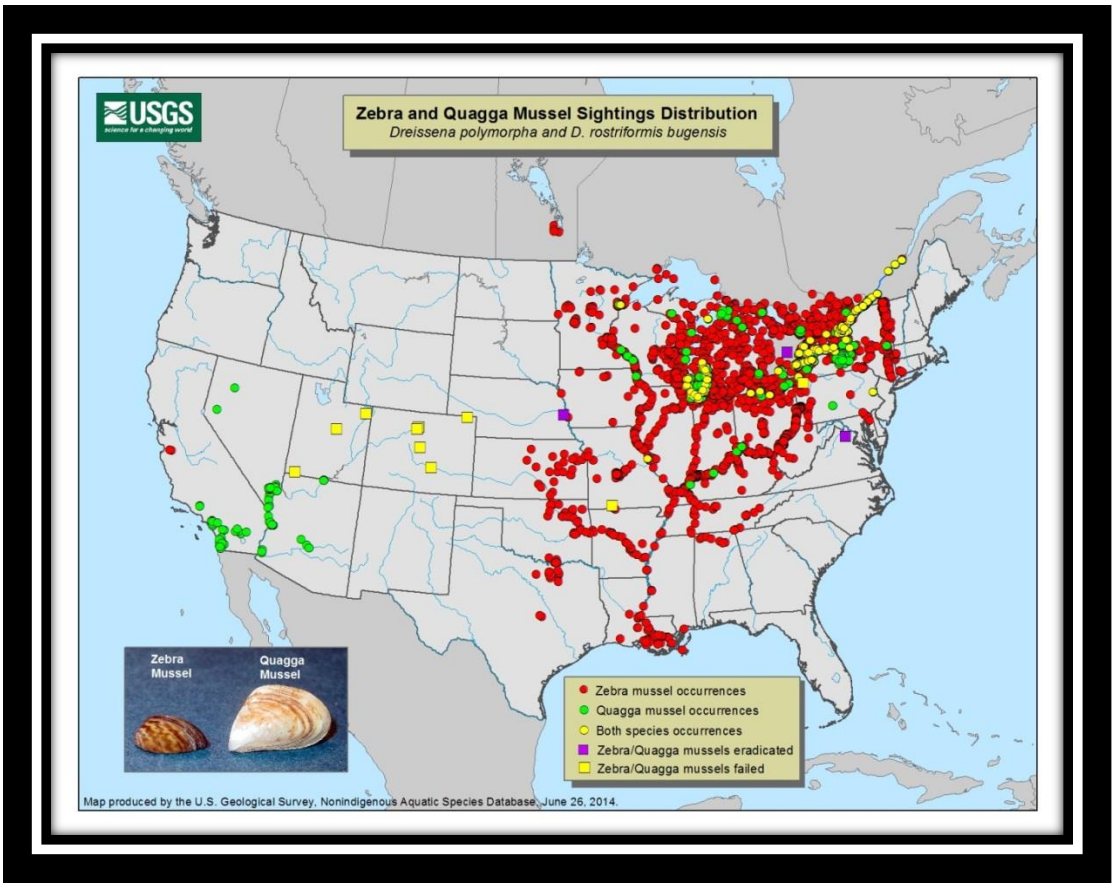


Figure 3. *D. rostriformis bugensis* and *D. polymorpha* distribution in the U.S. June 2014. Image from USGS, Nonindigenous Species Database. Retrieved [27 July 2014] from http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/maps/current_zm_quag_map.jpg

Figure 4. *D. rostriformis bugensis* and *D. polymorpha* distribution in the U.S. March 2007. Image from U.S. Fish and Wildlife Services (FWS). Retrieved [05 Apr 2014] from http://biologybiozine.com/mussels-wreaking-havoc-in-american-waterways/72

Even with mitigation and prevention programs in place at ports of entry of states or at the water body, mussels, particularly *D. rostriformis bugensis*, have continued to spread into California, Utah, and Arizona. As of January, 2014, the most recent water bodies tested to be positive for *D. rostriformis bugensis*, west of the 100th Meridian, are Lake Piru, located in Ventura County, California, in the Los Padres National Forest next to the Sespe Condor Sanctuary and Lake Powell, located in the Upper Colorado River system, straddling the border between Utah and Arizona.

Morphological Differences

Both *D. rostriformis bugensis* and *D. polymorpha* are epifaunal species that use byssal threads to live on and attach to substrates. Because of these similar modes of life, the two species have similar morphological features such as mytiloid shell form, shell allometry, variability in shell outline, and greater range in width/length than in height/length (Pavlova & Izyumov, 2014). Although the quagga and zebra mussel are similar in many respects, they are distinguishable from each other by certain external morphological differences, which are mostly related to shell proportions. *D. polymorpha* has a flat ventral margin which allows the mussel to remain upright when placed on a flat surface (Mills et al., 1996). *D. rostriformis bugensis* shells are less flattened on the bottom and are rounder, where the shell is higher and less convex than *D. polymorpha* shells. The *D. polymorpha* shell is more triangular, and the height and width are almost equal (Figure 5) (Marsden, Spidle, & May, 1996; Pavlova & Izyumov, 2014). Both *D. rostriformis bugensis* and *D. polymorpha* have permanent openings in which the byssal apparatus extends, and in the quagga mussel, this is more anterior (Claudi & Mackie, 1994). Additionally, *D. rostriformis bugensis* has 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, McCarthy, & Lee, 2009) than *D. polymorpha*.

Figure 5. Morphological differences between *D. rostriformis bugensis* and *D. polymorpha*. Image from U.S. GS. Retrieved [05 Apr 2014] from http://fl.biology.usgs.gov/Nonindigenous_Species/Zebra_mussel_FAQs/Dreissena_FAQs/zebra_quagga2.jpg

Shell pigmentation varies between *D. rostriformis bugensis* and *D. polymorpha*. There is great variability of patterns between the two species and the type of pattern may be indicative of the species' adaptation to the environment, age, or both (Pavlova & Izyumov, 2014). However, the internal morphology of *D. rostriformis bugensis* and *D. polymorpha* does not differ. Ciliary action is used to move water in the body cavity via the inhalant siphon. Digestible food particles move towards the mouth, and unpalatable particles are bound in mucus and then rejected via the inhalant siphon as pseudofeces (Crosier & Molloy, 2001). On the ventral side of the shell, the mussel has a muscular foot that is used for moving on a substrate and secretion of byssal threads (Claudi & Mackie,

1994). Alternate ways to identify dreissenid species is to analyze the DNA sequence through allozyme electrophoresis (May & Marsden, 1992; Spidle, Marsden, & May, 1994) or use Polymerase Chain Reaction (PCR) analysis of DNA sequences which can readily discriminate between dreissenid species in all life stages. The latter is quicker and less expensive, but could be less accurate (Stepien et al., 2014).

Physiological Tolerances

The morphology of *D. rostriformis bugensis* and *D. polymorpha* shows that upon initial observation they seem to be very similar; however, when examining the physiological tolerances such as temperature, dissolved oxygen, pH, calcium, salinity, and turbidity of each species, differences become apparent.

D. rostriformis bugensis and *D. polymorpha* are cold water tolerant and the incipient lower thermal limit is 0°C which allows both species to survive prolonged exposure in colder ranges so long as their surrounding waters do not freeze (Karateyev, Burlakova, & Padilla, 1998). Both species of dreissenids are generally intolerant of elevated temperatures beyond 30°C (Cohen, 2008). An incipient upper thermal limit tolerance for the two species is determined by multiple factors. These factors are based on the size of the mussel, nutrient availability, prior temperature experience, and the season (Elderkin & Klerks, 2005). The scientific literature generally shows that *D. rostriformis bugensis* have a lower thermal tolerance than *D. polymorpha* in North America. For example, a 28 day incipient upper thermal limit of *D. rostriformis bugensis* from Lake Mead, NV-AZ was 27.2°C, compared to 31.7°C for *D. polymorpha* from Winfield City, Kansas (Morse, 2009). Water temperatures above 25°C will lead to the eventual starvation of adult mussels. Water temperatures above 10 – 12°C are needed to

achieve spawning, although 18°C is likely to be optimal, while temperatures above 24°C will negatively impact mussel reproduction (Cohen, 2008).

D. rostriformis bugensis and *D. polymorpha* are relatively intolerant of low oxygen concentrations (range between 2-4 mg/L); however, *D. rostriformis bugensis* may tolerate levels as low as 1.5 mg/L (Cohen, 2008). Because of the intolerance to hypoxia, *D. rostriformis bugensis* and *D. polymorpha* are likely to be found and thriving in habitats that are well oxygenated; therefore, substrates in hypolimnetic regions are rarely colonized (Garton, McMahon, & Stoeckmann, 2014).

Currently, the literature is lacking studies that have evaluated the pH tolerance of *D. rostriformis bugensis* in Europe and North America. The studies that have examined the pH tolerances of *D. polymorpha* show that they are limited to alkaline waters. With a pH range 6.0-8.5, survivorship, shell growth, and successful development from fertilized egg to veliger was observed in *D. polymorpha* (Hinks & Mackie, 1997; Sprung, 1987). The estimated lower Ca^{2+} limits for *D. polymorpha* and *D. rostriformis bugensis* are 8.0 and 12.0 mg/L, respectively based on colonization and survivorship rates (Jones & Ricciardi, 2005). Minimum calcium requirements are difficult to determine for *D. rostriformis bugensis* and *D. polymorpha* because they are able to move Ca^{2+} from their shell to their hemolymph when necessary (Garton, McMahon, & Stoeckmann, 2014).

D. polymorpha are believed to be more tolerant of saline conditions when compared to *D. rostriformis bugensis*. Reported *D. polymorpha* tolerances to salinity vary widely (0.4 to 12 psu) and may be dependent on temperature, ionic makeup, and salinity stability of the reservoir (Garton, McMahon, & Stoeckmann, 2014). *D. polymorpha* have an upper salinity tolerance of approximately 12 psu, but byssogenesis is inhibited at ≥4

psu (Orlova, Khlebovich, & Kpmendantov, 1998). *D. rostriformis bugensis* may be less tolerant with an upper salinity tolerance of 6 to 8 ppt.

Turbidity can negatively impact metabolic functions of dreissenids by inhibiting oxygen consumption rates and cause starvation through decreasing filtration rates (Garton, McMahon, & Stoeckmann, 2014). Studies show that *D. rostriformis bugensis* may be better adapted to elevated turbidities than *D. polymorpha* because of the ability of *D. rostriformis bugensis* to sustain higher clearance and filtration rates (Summers et al., 1996; Diggins, 2001). Both species are able to adapt, but *D. rostriformis bugensis* seems to be more efficient.

There are similarities in the physiological tolerances of *D. rostriformis bugensis* and *D. polymorpha* because they are from the same genus. It is interesting to find that most research has been conducted on *D. polymorpha* and less is known about *D. rostriformis bugensis*. Having a better understanding of these physiological tolerances may allow for predicting further invasions and having the ability to possibly mitigate colonization.

Life Cycle and Reproduction

As mentioned previously, dreissenids rapidly invade new bodies of water because they are able to adapt to a wide range of habitats and by the flexibility of their reproductive cycle. Both *D. rostriformis bugensis* and *D. polymorpha* have a high rate of fecundity (Ram & McMahon, 1996). There are two phases of the dreissenid life cycle: sessile adult phase and the free-living larval phase (Figure 6) (Nichols, 1996). Adult mussels do not become sexually mature at a certain age, rather when they reach a shell length between 5 and 12 mm is when reproduction is likely to occur (Marsden, 1992).

Water temperatures are usually observed between 12°C and 15°C for *D. polymorpha* egg and sperm to be seen (Claudi & Mackie, 1994), but *D. rostriformis bugensis* has been seen to spawn at temperatures as low as 4.8°C (Roe & MacIsaac, 1997). A female mussel can have as many as 1,000,000 eggs per year (Neumann, Borcherding, & Jantz, 1993) and unlike other native bivalves, *Dreissena* release gametes into the water column where external fertilization of an egg and sperm occurs (Crosier & Molloy, 2001). Two days after fertilization, a trochophore (57-121 μm) develops (Nichols & Black, 1994). This is a rather short phase, and the trochophore metamorphoses into a veliger. A veliger (150-250 μm) is described as ciliated, free-swimming planktonic larvae which can be transported in water currents (Marsden, 1992). Two to nine days after fertilization, the veliger begins to secrete a D-shaped or straight hinged shell, which is still transparent (Crosier & Molloy, 2001). Within nine days, the shell has a more pronounced umbonal region near the hinges and is more rounded. The umbonal stage represents the last stage in which a veliger will be free swimming and found in the plankton (Marsden 1992; Claudi & Mackie, 1994). Two to three weeks after fertilization, the veliger is transitioning from the umbonal stage to the pediveliger stage (200-300 μm). The individual develops a velum (the organelle which facilitates in swimming) and a siphon; the foot lengthens and the organ systems begin to develop (Claudi & Mackie, 1994; Martel, 1993). Pediveligers are too dense to be carried with the water current, and after three to four weeks, they will settle onto substrates with the ability to crawl (3.8 cm/hr) around before extruding byssal threads, which allows them to attach to the substrate and become a plantigrade (> 500 μm) (Lewandowski, 1982; Marsden, 1992). This is the last phase before the veliger becomes a juvenile, and begins feeding with gills instead of a

velum, and moves solely with its foot (Crosier & Molloy, 2001). In the juvenile stage, the mussel has a more triangular or mussel-like shape and grows about 83-200 µm/week (Hincks & Mackie, 1997). It has been shown that *D. rostriformis bugensis* will grow at a faster rate than *D. polymorpha*. Given high food levels, quagga mussel growth was three times greater than zebra mussel growth at high temperature, and as high as 19 times greater than zebra mussel growth at a low temperature (Baldwin et al., 2002).

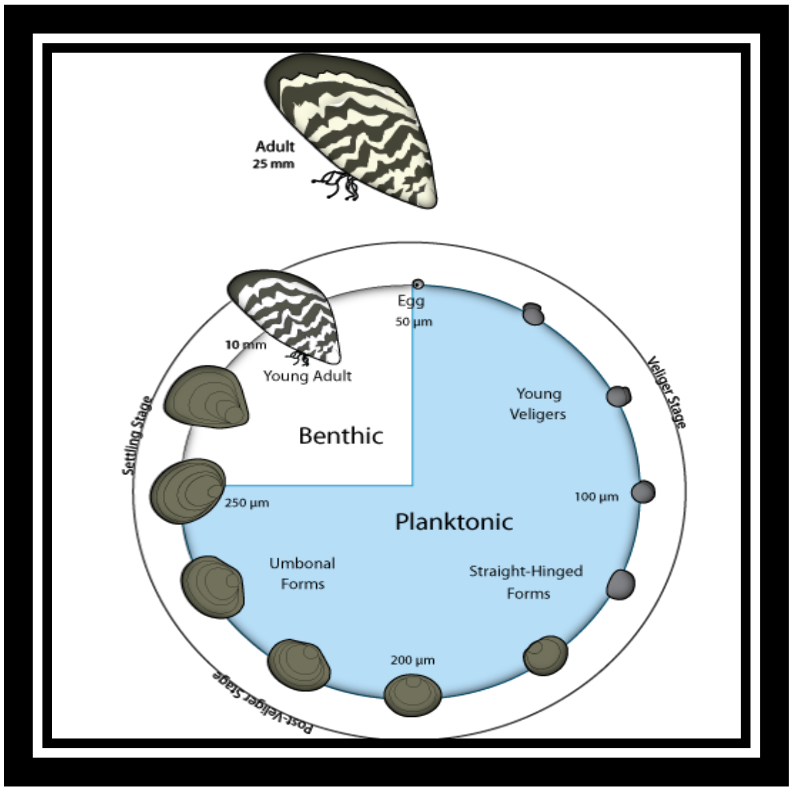


Figure 6. Life cycle of dreissenids from the free-living larval phase to the sessile adult phase. Image from the 100th Meridian Initiative. Retrieved [05 Apr 2014] from http://www.100thmeridian.org/

Settlement

D. rostriformis bugensis and *D. polymorpha* owe a large part of their success as invasive species to their ability to attach to a wide variety of substrates. Veligers will live in the water column for weeks before settling with proteinaceous byssal threads on a hard substrate, becoming a juvenile, and eventually forming a colony (Martel, 1993). The adhesive apparatus, also known as byssus is composed of adhesive pads that attach to the substrate, threads that attach to pads and connect to the stem, which holds the threads as a bundle and attaches them to the root (near the mussel shell) of the byssus (Farsad & Sone, 2012). The byssus is covered by a cross-linked protective coating composed of collagen-like proteins and a rare adhesive composed of 3,4-dihydroxyphenylalanine (DOPA) (Bonner & Rockhill, 1994; Lee, Messersmith, Israelachvili, & Waite, 2011). These strong components of the byssi of *D. rostriformis bugensis* and *D. polymorpha* allow them to tightly anchor themselves to substrates. Successful mussel colonies have been found on any natural surface from rock, benthic sediment, wood, aquatic plants and shells of other mussels, to man-made substrates such as boats, water intake pipes, marine infrastructure, buoys, and trash.

To mitigate and control colonization of *D. rostriformis bugensis* and *D. polymorpha*, it is important to understand the mechanism behind settlement. The shell shape of *D. rostriformis bugensis* and *D. polymorpha* is advantageous in that the flat, ventral surface allows the animal to be pulled tightly against the substrate by the byssal threads, which aids in the protection from predators. The umbone is adjacent to the substrate which gives the mussel upright stability at the surface of the substrate and the shell is tapered dorsally which makes it difficult for predators to pry the shell from the

substrate (Figure 7) (Claudi & Mackie, 1994). Zebra mussels can be found in large numbers at all depths of the epilimnion (3-7 m) but have been found as deep at 15 m (Claudi & Mackie, 1994). *D. polymorpha* have been found in the hypolimnion; however, the cold waters limit their growth and reproduction (Claudi & Mackie, 1994). In Lake Mead, *D. rostriformis bugensis* have been found over 108 m deep (Moore, Gerstenberger, & Wong, 2009). However, it is unclear if the mussels were able to reproduce at this depth. It has been shown that *D. rostriformis bugensis* settle at a larger size compared to *D. polymorpha*. Martel et al. (2001) explain that the mean size in settlement between the two species could be explained by a longer planktonic development time, ability to delay settlement, or a faster larval growth rate in *D. rostriformis bugensis*.

Figure 7. *D. rostriformis bugensis* attached with byssal threads to the hull of a watercraft

Numerous studies have been conducted to determine the type of substrate that *D. rostriformis bugensis* and *D. polymorpha* prefer and which type will deter their settlement (Martel, Mathieu, Findlay, Nepszy, & Leach, 1994; Wainman, Hincks, Kaushik, & Mackie, 1996; Marsden & Lansky, 2000; Aquatic Environmental Consulting, 2008). When settling on plates, mussels prefer stainless steel, polypropylene, black steel, pressure treated wood, Teflon, polyvinyl chloride (PVC), aluminum, and galvanized steel (Kilgour & Mackie, 1993). Based on the toxicity of copper, brass, and galvanized iron, mussels tend not to settle on these metals, and if they do, it usually takes longer to be colonized compared to other metals (Kilgour & Mackie, 1993). Suitable substrates for mussels are based on texture, chemical composition, orientation in the water, and presence of light and a biofilm (Kavouras & Macki, 2003). Marsden & Lansky (2000) found that zebra mussels prefer upper, horizontal surfaces versus lower surfaces, textured versus smooth surfaces, shaded versus sunlit surfaces, and plastics versus glass. *D. rostriformis bugensis* will settle on hard substrates, but unlike *D. polymorpha*, they will also colonize deeper waters and on softer substrates (i.e., soft sediment surfaces) (Mills et al., 1993).

D. rostriformis bugensis and *D. polymorpha* settlement rates are dependent on byssal thread strength. In laboratory settings, *D. polymorpha* showed the greatest byssal thread attachment strength to dolomite limestone and became weaker on other substrates such as PVC, stainless steel, aluminum, and acrylic glass (Ackerman et al., 1995). More research is needed to comparatively examine the strength of *D. rostriformis bugensis* byssal threads.

Under optimal conditions, 99% of veligers do not reach a suitable substrate on which to attach (Aquatic Environmental Consulting, 2008). Daily settlement rates are strongly correlated with the concentration of veligers found in the water column (Martel et al., 1994). Understanding *D. rostriformis bugensis* and *D. polymorpha* substrate preference can aid in controlling for the invasive species in vulnerable areas by using materials that deter settlement when building new infrastructure.

Vectors of Spread

There are numerous ways in which *D. rostriformis bugensis* and *D. polymorpha* spread from one water body to another. The primary mode in which *D. rostriformis bugensis* and *D. polymorpha* spread is through human activities such as intra-basin ballast water discharge, canal creations, waterway operations, and recreational boating (Johnson & Carlton, 1996; Ricciardi 2006). Other human driven vectors, which have a lower probability to spread *D. rostriformis bugensis* and *D. polymorpha*, include fish stocking, anglers, SCUBA gear, sea planes, and wildland firefighting equipment. The secondary mode of spread is through natural dispersal through drift or attachment to wildlife. As mentioned previously, dreissenids were brought to the North American Great Lakes through the discharge of ballast water, containing veligers, of large oceanic cargo ships. It is not likely that adult mussels fouling the hulls of these ships would be responsible for the infestation, as long transit times and oceanic environments exceeding salinity tolerances would be detrimental to the survivorship of the mussels (Therriault et al., 2013).

Transient recreational boating activity is suspected of being the primary means of overland dispersal and several mechanisms associated with boating have been shown to

be capable of transporting mussels in large numbers (Johnson & Padilla, 1996). Any activities that can move water containing veligers or those that contain attached mussels within or between bodies of water has the potential to accelerate the spread of dreissenids, especially upstream or overland.

Adult mussels are thought to have a greater potential to establish new populations in un-infested water bodies as opposed to veliger transfer from incidental release from recreational vessels (Johnson & Padilla, 1996). Mussels can encrust the watercraft's hull, engine, anchor lines, and the microscopic larvae can be found in standing water of the bilge, ballast tanks, and in live/bait wells (Figure 8). Adult mussels and veligers can also be transported on aquatic vegetation entangled on the trailer or boat exterior.

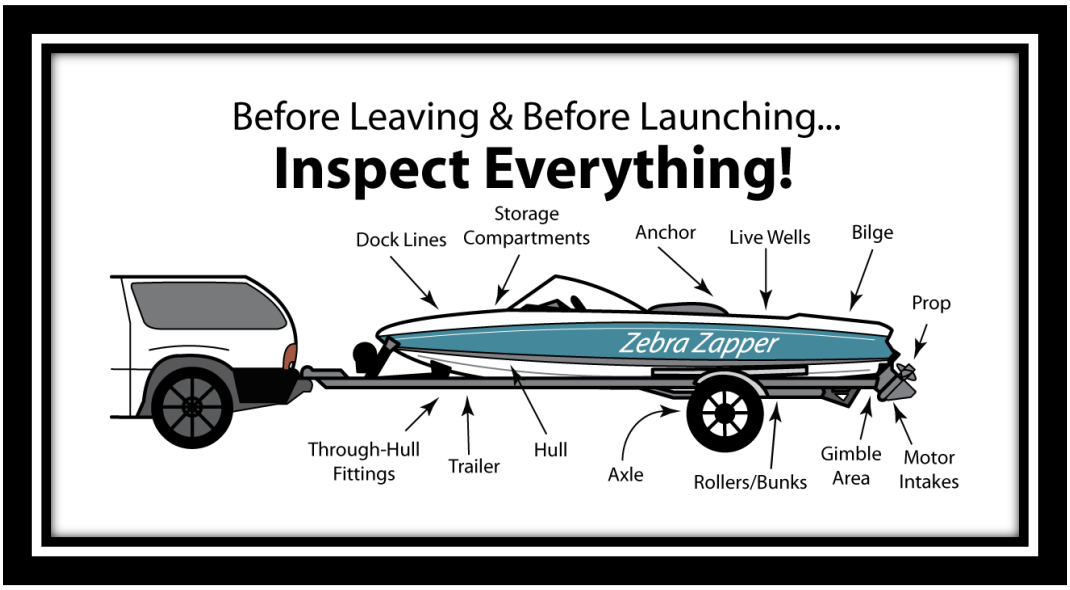


Figure 8. Common areas where *D. rostriformis bugensis* and *D. polymorpha* adults and veligers are found on watercraft. Image from the 100th Meridian Initiative. Retrieved [05 Apr 2014] from http://www.100thmeridian.org/

Survivorship of mussels exposed to the air, increases with increasing relative humidity, decreasing temperature, and increasing mussel size. During overland transport, adult *D. polymorpha* can survive approximately 3-5 days under temperate summer conditions when transported overland on small trailered vessels; however, desiccation can be reduced if the mussels are attached to moist aquatic plants (Ricciardi, Serrouya, & Whoriskey, 1995). The maximum survival rate of adults is estimated at approximately three weeks under conditions of low temperatures, <5°C and high relative humidity ≥95% (McMahon, 1996). Adult *D. rostriformis bugensis*, from Lake Mead, NV-AZ, can survive for less than one day in hot conditions 30ºC or higher and in cooler, more humid conditions, adult *D. rostriformis bugensis* can survive longer than five days (Kappel, 2012).

D. rostriformis bugensis and *D. polymorpha* veligers are a great threat to un-infested water bodies because they are microscopic; therefore, more difficult to detect and are more commonly transported than adults because of their occurrence in the water body that accumulates in a variety of places on the vessel. Because they are the most vulnerable stage of the mussel, they are more susceptible to outside environmental conditions which contribute to increased mortality compared to adults. However, veligers can be found in the vessel ballast tanks, bilge compartments, and in engine cooling water.

Bilge compartments and ballast tanks are enclosed areas and usually located beneath the deck of the vessel. They provide a more suitable environment for veligers to travel greater distances, as they allow protection for the organisms from UV radiation and maintain the static water at a cooler temperature (Kelly, Wantola, Weisz, & Yan, 2013).

While the veligers may be protected from the environmental conditions, they are still exposed to the contaminants which commonly drain into the bilge compartment, such as oil, fuels, and metals (Penny & Suominen-Yeh, 2006). Currently, there is no published data that have examined the survivorship of veligers drained from bilge compartments.

Some vessels, such as wake board boats, pump gallons of water straight from the reservoir into ballast tanks, which are used to stabilize the watercraft. *D. polymorpha* veligers can survive ballast water transport for 11-15 days at 12-24°C, which could be extended should the optimal conditions be in place (Pollux, Van der Velde, & Bij de Vaate, 2010). Field tests demonstrated that *D. rostriformis bugensis* veligers, from Lake Mead, NV-AZ, can survive in contained water for five days in summer and for 27 days in autumn (Choi, Gerstenberger, McMahon, & Wong, 2013).

Veligers can also be transported in the bait and live wells of the vessel. Conditions in these compartments can be degraded by fish excretions, thus limiting the survivorship of the veligers (Johnson, Ricciardi, & Carlton, 2001). However, if fish are not caught, or their time is limited in the well, viable veligers can be transported. Veligers can also be found in the water of the engine cooling system. Water becomes trapped in the intake of the engine cooling system where temperatures are not elevated. The engine cooling system is likely to be the most suitable area of veliger survivorship (Johnson, Ricciardi, & Carlton, 2001). The engine cooling system holds approximately one liter of water and that small volume suggests the number of transported veligers to be low (Figure 9). This area of the engine can be challenging to completely drain and dry as direct sunlight does not go that far into the engine, nor is there a plug.

Figure 9. Vessel out of the water for several weeks showing water leaking from engine

Recreational vessels are not only responsible for overland dispersal, but they are also a vector for spreading *D. rostriformis bugensis* and *D. polymorpha* upstream. Vessels can move through inter-connected waterways. In-water transport may promote longer species survival during transport, thus increasing the likelihood for dispersal and succeeding establishment (Kelly, Wantola, Weisz, & Yan, 2013). Because of the harsh environmental conditions mussels may encounter during overland dispersal, their likelihood of survivorship is reduced; however, the vessels travelling in the water tend to travel over shorter distances, increasing the probability of the veligers being released alive into a new waterway.

Other human driven vectors, which have a lower potential to spread *D. rostriformis bugensis* and *D. polymorpha*, include fish stocking, anglers, SCUBA gear,

sea planes, and wildland firefighting equipment. While the probability of dreissenid spread is lower when compared to recreational boating, these possible vectors are being considered more by the aquatic invasive species community.

Wildland firefighting equipment moves large volumes of raw water during fire incidents to extinguish flames or control fire growth, and the water may serve as a pathway for *D. rostriformis bugensis* and *D. polymorpha* to be moved between water bodies. The equipment used may become contaminated and serve as vectors for future invasions across large geographic areas. *D. rostriformis bugensis* and *D. polymorpha* pose a risk to firefighting equipment, including water tanks, pumps on fire engines, portable pumps (water pumps that will suck water from lakes, streams, portable tanks, and ponds and then pump the water to a fire engine or water tender), backpack sprayers (worn by the ground crew to spray water on the fire), water tenders (large trucks that can transport >1000 gallons of water close to the fire), portable tanks, helicopter buckets (hang from the helicopter and pick up water directly from the reservoir), and fire engines (draft water directly from the reservoir) (Figure 10).

Figure 10. Examples of firefighting equipment: A) backpack sprayer, B) water tender, C) portable tank, D) portable pump, E) helicopter bucket, and F) fire engine (U.S. Forest Service, 2012)

If equipment is not completely drained or decontaminated and dried, un-infested water bodies, including remote and isolated headwaters, can be infested (Britton & Dingman, 2010). There is increasing recognition that firefighting operations can be disrupted by equipment fouled by *D. rostriformis bugensis* and *D. polymorpha* and that the enormous volumes of raw water moved during firefighting operations poses a risk for trans-basin transfer of mussels. This risk is compounded by the reality that firefighting equipment is highly mobile both within a single incident and between incidents, thus equipment contaminated at one incident may be dispatched immediately to an incident in a different state and serve as a vector for long distance dispersal with a speed not commonly seen in recreational vectors (e.g., a piece of fire equipment contaminated with *D. rostriformis bugensis* veligers in the Lower Colorado River could be dispatched to a fire in the Columbia River Basin, which is un-infested, and arrive on scene within a few hours while veligers are still viable).

D. rostriformis bugensis and *D. polymorpha* also have the capability to disperse via natural mechanisms such as drift and attachment to wildlife. After the initial

infestation of *D. rostriformis bugensis* and *D. polymorpha* in the Great Lakes, most of the range of the mussels was within lakes, rivers, and waterways that were directly connected. Canal creations, such as the Erie Canal, were able to connect more waterways and enhance the spread of the mussels (Johnson & Padilla, 1996). Range expansion downstream occurred at a fast rate, and by the end of 1993 *D. polymorpha* were distributed from Quebec to Louisiana, and adult mussels had only been found in eight isolated inland lakes (i.e., lakes without navigable connections with infested waters and with no reported populations of *D. polymorpha* upstream) (Johnson & Carlton, 1996).

Aquatic animals such as turtles, muskrats, and waterfowl can be considered potential vectors in spreading *D. rostriformis bugensis* and *D. polymorpha* to un-infested water bodies, but the likelihood of a successful population being established is low. The transport of *D. polymorpha* by waterfowl has been examined experimentally, and although waterfowl are capable of transporting small numbers of larval and juvenile stages (<0.5 zebra mussel/bird), the numbers appear insignificant relative to those of other vectors, such as recreational vessels (Johnson & Carlton, 1996).

Control Methods

Control strategies for *D. rostriformis bugensis* and *D. polymorpha* are typically categorized as preventative, proactive, and reactive (Chakraborti, Madon, Kaur, & Gabel, 2014). Preventive controls are intended to prevent the establishment of mussels through public education and outreach, vessel inspections, and regulations (local, state, or federal). The main objective of proactive treatment is to inhibit attachment of veligers or the translocation of adult mussels to other parts of infrastructure, such as water intakes and pipes. Techniques include chemical treatment, antifouling or foul-release paints on

infrastructure, mechanical in-line strainers and filters, and UV radiation (Macki & Claudi, 2010). Reactive control approaches are used when *D. rostriformis bugensis* and *D. polymorpha* become abundant and widely established in a water system (Chakraborti, Madon, Kaur, & Gabel, 2014). Reactive control methods include chemical treatments, mechanical removal of mussels using power wash and scraping, thermal shock or freezing, desiccation, and oxygen deprivation (Mackie & Claudi, 2010). There are numerous ways to combat a mussel problem; however, rarely are these techniques used alone, rather they are used by combining methods.

Mechanical cleaning involves using mechanical scrubbers or high pressure to remove mussels from all external structures and large diameter piping (Claudi & Mackie, 1994). This may not be the ideal method because the pipeline is unavailable during cleaning and the structures may not be able to withstand the pressure generated by the scrubbers. Once the mussels are knocked off the pipe walls, a large amount of mussel debris has been created and those mussels will need to be removed and disposed. High pressure can be used to remove fouling from vessels and marine infrastructure such as docks and barges.

Methods using hot water spray have been proven effective against *D. polymorpha* in the laboratory setting and *D. rostriformis bugensis* in both the laboratory and field settings within minutes (Morse, 2009; Comeau et al., 2011). Comeau et al. (2011) found that *D. rostriformis bugensis* exposed to hot water (>60°C) for 5 s is sufficient to reach 100% mortality. Power plants or industries where excess heat is available to raise water temperatures have the advantage to combine chemical and heat control strategies. For example, the addition of chlorine at elevated temperatures can reduce mortality times of

D. polymorpha by as much as three orders of magnitude compared to oxidant addition at ambient temperatures (Harrington, Van Benschoten, Jensen, Lewis, & Neuhauser, 1997).

As mentioned previously, *D. rostriformis bugensis* and *D. polymorpha* can live from 3-21 days out of the water depending on ambient temperature and humidity (McMahon, Ussery, & Clarke, 1993). Using desiccation as a mechanism for *D. rostriformis bugensis* and *D. polymorpha* control can be time consuming and costly. The entire facility would need to be shutdown to drain the pipes and allow the mussels to dry out (Claudi & Mackie, 1994). It would be advantageous to use hot air to heat the pipes to speed up the process.

Another option for controlling *D. rostriformis bugensis* and *D. polymorpha* is through biological controls. There are limited biological control methods that are available and efficient in either deterring mussel settlement or killing mussels. Dead cells from naturally occurring bacteria, *Pseudomonas fluorescens,* are found to cause death in dreissenids by disrupting the epithelial lining of the digestive tract post ingestion (Molloy & Mayer, 2007). *P. fluorescens* is present in soil and water, and is naturally known to protect plants from diseases. The mode of action of *P. fluorescens* is intoxication, not infection. Mussel deaths occur following lysis and necrosis of the digestive gland and sloughing of the stomach epithelium (Molloy et al., 2013). *P. fluorescens* is patented as a molluscicide under the product name Zequanox®, and the U.S. Environmental Protection Agency has approved its use for managing dreissenid infestations in lakes and rivers. It has minimal risk to humans and non-target species, requires minimal personal protective equipment, it is noncorrosive and nonvolatile, and uses short treatment times (Zequanox®, 2012).

There are numerous chemical control methods that may be effective in controlling *D. rostriformis bugensis* and *D. polymorpha*. Following the introduction of *D. rostriformis bugensis* and *D. polymorpha*, a number of chemicals with unknown and known molluscicidal properties have been proposed for use in controlling these invasive species (Sprecher & Getsinger, 2000). Regardless of the chemical being used, it must be cost effective, nontoxic to the surrounding aquatic ecosystem, and safe as an additive in drinking water.

Methodology is as important as what chemical is chosen. Application strategies are also important to follow when administering a chemical or toxicant. There are five basic ways to apply a chemical treatment: end of season, periodically, intermittently, semi-continuously, and continuously (Claudi & Mackie, 1994; Sprecher & Getsinger, 2000). The end of season treatment is applied at the end of the breeding season to kill adult mussels that are established within the water system (Sprecher & Getsinger, 2000). As a result of this method of application only being applied once a year, dead mussel debris can build up and this may be a problem for water treatment facilities. Periodic treatment is similar to end of the year treatment, but is done more frequently. While adult mussels are still the target, periodic treatment may also be effective in preventing new settlement of juveniles if administered frequently enough (Claudi & Mackie, 1994; Sprecher & Getsinger, 2000). Intermittent, semi-continuous and continuous treatments are all designed to prevent new settlement of mussels in raw water systems. Oxidizing chemicals work best with intermittent treatments at frequent intervals (i.e., every 6, 12, and 24 h) (Claudi & Mackie, 1994). The aim is to destroy post-veliger stages of development and to prevent further mussel infestation. Semi-continuous treatment creates

a constant state of stress in mussels. The treatment schedule can be adjusted to 15 min on and then 45 min off, effectively controlling all stages of mussels in the piping systems. Continuous treatment is used when a low concentration of a chemical can be used continuously. It is typically used in systems that cannot tolerate any biofouling, such as fire protection systems (Claudi & Mackie, 1994).

Chlorination is currently the most commonly used chemical control method for *D. rostriformis bugensis* and *D. polymorpha* in water treatment facilities and hydropower plants. The widespread use of chlorine is based on its effectiveness as a molluscicide, presence in water treatment facilities, and its use and side effects are generally well understood by operators and regulators (Chakraborti, Madon, Kaur, & Gabel, 2014). The benefits of chlorine are that it is effective at low concentrations and efficient against all fouling categories ranging from bacteria to mollusks. It not only kills adult *D. rostriformis bugensis* and *D. polymorpha*, but is effective in preventing veligers from settling in raw water piping systems, thus increasing the water facility's efficiency (Jenner & Janssen-Mommen, 1993). Chlorine controls mussels through an oxidation process either directly on the adults or through inhibition of settlement and growth of the veligers. It damages the membranes by diffusing through the cell wall and disrupting enzyme activities (Claudi & Mackie, 1994). Mussels are able to sense chlorine in low doses when it is present in the water. They will react by closing their valves, and cease filter feeding, making it necessary to survive off stored food reserves and anaerobic respiration (Rajagopal, van der Velde, & Jenner, 1997; Rajagopal, van der Velde, & Jenner, 2002). Because mussels try to avoid the chemical, they may actually die from

asphyxiation or limited glycolysis over a prolonged period of time (Van Benschoten, Jensen, Harrington, & DeGirolama, 1995).

A major concern with the use of chlorine is the development of trihalomethanes (THM). These by-products are formed when chlorine reacts with organic or inorganic material already present in the water being treated. THM are halogenated single carbon compounds that include chloroform, bromodichloromethane, dibromochloromethane, and bromoform. THM are linked to adverse health effects and have been shown to be carcinogenic to animals (Cotruvo & Regelski, 1989). The U.S. EPA has set a standard for the maximum allowable annual average concentration level of total THM of 80 ppb (U.S. EPA, 2010). In cases where THM exceeds the U.S. EPA's limit, an alternate form of chemical control should be implemented.

Chloramine is a family of organic compounds with the formulas R_2NCl and $RNCl_2$ that may be a suitable alternative to chlorine when THM concentrations become too high. Chloramines are formed naturally when free available chlorine reacts with nitrogen compounds, such as ammonia and amino acids. Low doses of chloramine compounds result in a high rate of veliger mortality in both static and flow-through tests (Van Benschoten et al., 1993). The Southern Nevada Water Authority has recently switched from using chlorine to chloramine to disinfect drinking water and to deter veliger settlement. Disadvantages to using chloramine include safety considerations, constructing new facilities for handling and preparations, and they may not be compatible with current disinfection systems (Chakraborti, Madon, Kaur, & Gabel, 2014).

Nonoxidizing chemicals such as potassium, copper, and quaternary ammonium compounds are generally more cost effective, relatively inert to system metallurgies and

materials, environmentally acceptable, and incapable of producing potential carcinogenic by-products when compared to oxidizing chemical controls such as chlorine. These chemicals are toxic to bivalves in low concentrations, readily inactivated, and application and storage are generally easier to handle (McMahon, Shipman, & Long, 1993). Potassium and copper are both effective chemical control methods against quagga and zebra mussels when used in closed systems; however, they can be toxic to the surrounding aquatic ecosystem and are not used as often (McMahon, Shipman, & Long, 1993).

Quaternary ammonium compounds, commonly referred to as Quats, are commonly used as disinfectants, surfactants, fabric softeners, and antistatic agents in shampoos and conditioners (Patrauchan & Oriel, 2003). They are common cleaning agents used in homes, workout equipment at gyms, swimming pools, daycare centers, and hospitals. These compounds are relatively nontoxic, when compared to other oxidizing chemicals, such as chlorine, as they do not damage fabric, metals, or gaskets, and are easy to acquire from local stores (U.S. Forest Service, 2012). The U.S. Forest Service and other firefighting agencies have been prescribing the use of quaternary ammonium compounds as decontaminants to prevent the spread of AIS such as whirling disease, chytrid fungus, didymo, and New Zealand mud snails for fire operations (Southwest Geographic Coordinating Group 2009; U.S. FS 2012).

Quats are a large and complex group of compounds that have numerous uses and are available in a large number of formulations. They are composed of four organic groups linked to a nitrogen atom that produces a cation including alkyl dimethyl benzylammonium chlorides (ADBAC) and didecyl dimethyl ammonium chloride

(DDAC), and most are sold under various product names including Roccal, Germex, and San-O-Fec (U.S. EPA, 2006a; U.S. EPA, 2006b). The active ingredient in Quat™ 128 and Quat™ 256 is composed of a blend of DDAC (5.07%) and ADBAC (3.38%) and DDAC (10.14%) and ADBAC (6.76%), respectively. Unlike ammonium, the quaternary ammonium cations are permanently charged independent of the pH and are stable with a long shelf life. The chemical structure allows the compound to bind to organic and inorganic surfaces (Rahn & Van Eseltine, 1947).

CHAPTER 3

METHODOLOGY

Pressurized Water Spray to Remove Dreissenids on Watercraft

High pressure water spray for watercraft decontamination has been used in practical settings, such as state ports of entry, national park areas, and at private bodies of water; however, there is no systematic study on this topic that has validated the data as a basis for sound recommendations for standard watercraft and equipment decontamination protocols for the Western U.S.

Research Objectives

1. To examine the relationships between *D. rostriformis bugensis* and *D. polymorpha* removal and water spray pressures and exposure duration
2. To determine the minimum amount of time required to achieve 100% removal rate at 1500 and 3000 psi of water by decontaminating watercraft infested with *D. rostriformis bugensis* and *D. polymorpha*
3. To determine the season, winter or summer, when *D. rostriformis bugensis* and *D. polymorpha* are more efficiently removed from watercraft when using 1500 and 3000 psi of water
4. To provide data for the development of a standard protocol using pressurized water spray for removing 100% of *D. rostriformis bugensis* and *D. polymorpha* attached to watercraft within the Western U.S.

Methods

Quagga Mussels

The effectiveness of high pressure water spray (1500 and 3000 psi of water) was evaluated for removing 100% of *D. rostriformis bugensis* from watercraft in the winter season and then repeated in the summer season at Lake Mead NRA.

A heavily encrusted Bayliner watercraft, which was slipped in Lake Mead for over four years, was pulled from a Las Vegas Boat Harbor slip, on January 28, 2011 and brought to the maintenance yard (Figure 11). The mussels on the watercraft, pulled fresh out of the water, were presumed alive. That same watercraft remained in the maintenance yard where the experiment was repeated on weeks 2 (February 11, 2011) and 4 (February 27, 2011). Another Bayliner was pulled from a slip at Las Vegas Boat Harbor on July 20, 2011 for the summer season, high pressure experiment. It was brought back to the marina's maintenance yard where the experiment took place on weeks 0 (July 20, 2011) and 1 (July 27, 2011).

Figure 11. Watercraft heavily encrusted with *D. rostriformis bugensis*

Groups of mussels were divided into 24 treatment groups: 2 pressures (1500 and 3000 psi) x 2 densities (high and low) x 6 replicates (Table 1). The treated area was completely covered with mussels and was created by partitioning off areas (high or low density mussel groups) by scraping off the surrounding mussels (Figure 12). High mussel density groups consisted of approximately 23,220-46,440 mussels/m^2 (~75-150 individuals) and low density mussel groups consisted of approximately 7,772-10,363 mussels/m^2 (~15-20 individuals). Prior to using pressurized spray, each group of mussels was photographed for a more precise enumeration. A LANDA pressure washer (LANDA Cold Water Direct Drive Pressure Washer, Model # PD4-35324; American Pressure Inc., Robbinsdale, MN) was used for the high pressure experiments (Figure 13). The unit is capable of spraying at least 5 gallons/minute with a nozzle pressure of 3000 psi of water and greater, which is recommended by The Uniform Minimum Protocols and

Standards for Watercraft Interception Programs for Dreissenid Mussels in the Western U.S. (Zook & Phillips, 2012). For this project, the 40 degree nozzle was used and the tip of the pressure washer wand remained 12 inches away from the watercraft. A sub-sample of mussels was removed from each replicate, and the size of mussels was recorded. The shell length of the mussel is the distance measured from the posterior edge of the shell to the anterior tip of the umbos to the nearest 0.1 mm with digital calipers (VWR Digital 152 cm (6") Caliper - Stainless Steel, Model # 62379-531; VWR International, Inc., Miamai, FL) (McMahon & Ussery, 2005).

Table 1. Experimental design for pressurized water spray to remove *D. rostriformis bugensis* and *D. polymorpha* on watercraft

Mussel Density	1500 psi	3000 psi
High	6 replicates	6 replicates
Low	6 replicates	6 replicates

Figure 12. A group of mussels segmented for high pressure testing

Figure 13. LANDA pressure washer used in the high pressure experiments

The above experiment was repeated in the summer season at the Las Vegas Boat Harbor with one modification. The summer season experiment took place on weeks 0 (when the watercraft is pulled fresh out of the water) and 1. The shorter time range is because *D. rostriformis bugensis* byssal threads will dry out and decompose at a faster rate in the hot, arid days of summer in the Southwest compared to the winter months.

Zebra Mussels

The above study was repeated using *D. polymorpha* at Wilson Lake, Kansas during the summer season. Adult *D. polymorpha* colonies are not found in bodies of water in the southwest. Kansas is the closest place to Nevada, where the reservoirs contain healthy populations of adult *D. polymorpha*. In August 2011, the LANDA pressure washer and all other equipment were transported by van, to Wilson Lake, Kansas to conduct the experiment.

Arrangements were made with the marina staff at Wilson Lake to have a *D. polymorpha* encrusted watercraft removed from the reservoir to perform the project. Marina staff discovered they had a sunken boat lift that was encrusted with larger *D. polymorpha* compared to the watercrafts that were in the slips. The staff believes that the boat lift was in the water for over two years. It was removed from the reservoir and used for the high pressure experiment (Figure 14). On weeks 0 (August 1, 2011) and 1 (August 8, 2011), 1500 and 3000 psi of water was applied on high and low mussel densities to evaluate the amount of time it would take to remove 100% of the mussels.

Figure 14. Boat lift used for the summer season, Wilson Lake high pressure experiments

Statistical Analysis

Analysis of covariance (ANCOVA) was used to test the efficacy of removing *D. rostriformis bugensis* and *D. polymorpha* under different pressures and duration. The dependent, independent, and covariate variables are mussel density, pressure, and duration, respectively. The significance criterion was set at alpha = 0.05. All the statistics were performed using SAS® (Version 9.3, SAS Institute Inc. Cary, NC, U.S.A.).

Susceptibility of Zebra Mussels to Hot-water Spray

A recent project examined the susceptibility of *D. rostriformis bugensis* to hot-water sprays at different temperatures and durations of spray contact at Lake Mead NRA. Results showed that a spray temperature of 60°C for 5 s is recommended for mitigating fouling by *D. rostriformis bugensis* (Comeau et al., 2011). Currently, there are no studies in the literature validating this recommendation for *D. polymorpha*.

Research Objectives

1. To determine the temperature and exposure time needed to attain 100% mortality of *D. polymorpha* following exposure to hot-water spray
2. To determine which species of dreissenids, *D. rostriformis bugensis* or *D. polymorpha*, are more susceptible to hot-water spray
3. To validate the results of the susceptibility of *D. polymorpha* to hot-water sprays on watercraft
4. To provide data in the development of a standard protocol for killing *D. rostriformis bugensis* and *D. polymorpha* using hot-water spray in the Western U.S.

Methods

Specimen Collection and Experimental Design

A Kansas Department of Wildlife permit was obtained to collect adult *D. polymorpha* from Wilson Lake (APPENDIX A). Specimens of healthy adult *D. polymorpha* (≥11 mm in length) were collected from the encrusted docks at the marina at Wilson Lake, Kansas. The individuals were divided among 60 mesh spat bags

(approximately 75 in each) and suspended in the lake, off the dock, and acclimated for ten days. After acclimation, adult mussels were randomly divided into 60 subsamples (n = 50) and placed into 60 identical pre-labeled, 3 mm spat bags (Aquatic Eco-Systems Inc., Apopka, FL) (Table 2). To avoid transporting the mussels, the experiment took place on the dock, close to an electrical outlet to plug in the equipment. Each bag was suspended over a programmable heated circulator water bath with a 28 liter capacity during the thermal spray treatment (PolyScience, Model # 1137-2P; Niles, Illinois) (Figure 15). Treatment spray was applied to the samples at a flow rate of approximately 900 ml/min through a fan shaped nozzle (Comeau et al., 2011).

Table 2. *D. polymorpha* tested per treatment group (n = 50) (Comeau et al., 2011)

Temp °C/°F	Exposure Duration (s)							
	1	2	5	10	20	40	80	160
20/68	50	50	50	50	50	50	50	50
40/104	50	50	50	50	50	50	50	50
50/122	50	50	50	50	50	50	50	50
54/130	50	50	50	50	50	50	50	50
60/140	50	50	50	50	50	50	50	50
70/158	50	50	50	50	50	50	50	50
80/176	50	50	50	50	50	50	50	50

Figure 15. PolyScience programmable heated circulator wash bath

Each sample of mussels were exposed to thermal-spray treatments from a distance of 15 cm horizontally above the mussel-containing mesh bag (Morse, 2009) at 20, 40, 50, 54, 60, 70, and 80°C and exposure durations of 1, 2, 5, 10, 20, 40, 80, and 160 s. The control was 20°C. Therefore, 56 combinations of temperature by exposure duration were tested (Table 2). The water temperature, on contact with the treatment group, was constantly monitored by the programmable heated circulator water bath. Four bags, containing *D. polymorpha*, were treated with hot-water spray as they were used as controls and were left suspended in Wilson Lake. Following treatment, each spat bag containing the treatment specimens was tied to a line hanging from the dock. Mortality was assessed at the time of treatment and every day thereafter for ten days. To test for mortality, gaping mussels were gently prodded on their shell valves. Individual mussels that did respond by immediate shell closure were stimulated in the area of their siphons.

Mussels that did not respond to siphon stimulation had their shell valves forcible closed with forceps. Mussels were considered dead if the shell immediately reopened upon release of the forceps (Harrington et al., 1997; Morse, 2009; Comeau et al., 2011). Dead mussels were removed and measured, then recorded and placed into a different labeled mesh bag. The control groups remained in Wilson Lake for ten days and survivorship was assessed as described previously.

Hot-water Spray Watercraft Validation

After the minimum time to kill 100% *D. polymorpha* was identified from the above experiment, the protocol was field validated using a zebra mussel encrusted Crestliner pontoon vessel from Wilson Lake, Kansas. The watercraft was pulled from the reservoir on August 3, 2011. Five groups of mussels were segmented on the watercraft (as discussed previously) and served as the treatment groups. An additional group was segmented and served as the control, and those mussels were gently scraped off the watercraft, transferred to a mesh bag, and placed in the lake for ten days to assess survivorship. A heated circulator water bath was set at 54°C, which was the temperature that was discovered to kill 100% of *D. polymorpha* in the previous laboratory experiment. The spray nozzle was placed 15 cm from the treatment groups. The hot-water spray was applied to each group for 10 s. Once the *D. polymorpha* were treated with the hot-water spray, the mussels were gently scraped off the watercraft, transferred into a labeled mesh bag, and placed back into the lake to confirm mortality after 24 h and for ten days thereafter.

Statistical Analysis

The data were modeled and analyzed to find the minimum duration time to result in 100% *D. polymorpha* mortality using hot-water spray. ANCOVA was used to test the efficacy of killing *D. polymorpha* at different temperatures and durations. The dependent, independent, and covariate variables were mortality, temperature, and duration, respectively. A two-way analysis of variance (ANOVA) was used to examine if there were any significant difference in shell length at different temperatures with different exposure durations. The significance criterion was set at alpha = 0.05. All the statistics were performed using SAS® (Version 9.3, SAS Institute Inc.).

The Efficacy of Quaternary Ammonium Compounds on Killing Dreissenids

Quats are common cleaning agents used in homes, schools, gyms, and hospitals. Because they are relatively nontoxic and do not damage fabric, metals, or gaskets, the U.S. Forest Service and other firefighting agencies have been using Quats, as a decontamination method to prevent the spread of AIS (U.S. FS, 2012). The present study looked at the effectiveness of Quat™ 128 [active ingredient is composed of a blend of DDAC (5.07%) and ADBAC (3.38%)] and Quat™ 256 [active ingredient is composed of DDAC (10.14%) and ADBAC (6.76%] on killing dreissenid adults and veligers.

Research Objectives

1. To examine the relationships between the mortality of *D. rostriformis bugensis* and *D. polymorpha* adults and concentration of Quat™ 128 and Quat™ 256 during different treatment times

2. To determine the minimum amount of time required to reach 100% mortality of *D. rostriformis bugensis* and *D. polymorpha* veligers at different concentrations
3. To test the effectiveness of Quat™ 128 and Quat™ 256 at different water temperatures as well as the strength of the solution exposed to different ambient temperatures
4. To provide baseline data on the development of a standard and effective decontamination protocol on firefighting equipment exposed to quagga and zebra mussels throughout the Western U.S.

Methods

Specimen Collection

This project took place at Lake Mead NRA and Wilson Lake, Kansas. A National Park Service permit (APPENDIX B) was obtained to collect adult and veliger *D. rostriformis bugensis* at Lake Mead, and a Kansas Department of Wildlife permit was obtained to collect adult and veliger *D. polymorpha* in Wilson Lake. Adult *D. rostriformis bugensis* (500 individuals) were collected off the dock at the Las Vegas Boat Harbor in Lake Mead NRA (36°1'50.69"N; 114°46'12.95"W). The mussels were brought back to the Nevada Department of Wildlife's (NDOW) hatchery in Boulder City, Nevada to acclimate for five days, in aquaria which were stocked exclusively with water pumped directly from Lake Mead and equipped with a flow through system and aeration for the mussels. Adult *D. polymorpha* (500 individuals) were collected off the marina dock at Wilson Lake (38°54'51.3"N; 98°29'50.95" W). They were divided into mesh bags and suspended off the dock, in lake water for five days for acclimation. Following acclimation, *D. polymorpha* were brought back to the laboratory in Wilson, Kansas for

experimentation. For these tests, dreissenids greater than 11 mm were considered adults, and any mussel less than 11 mm was considered a juvenile and not used. Extra mussels were collected to ensure only alive mussels were used after acclimation.

D. rostriformis bugensis veligers (N = 1200) were collected from Lake Mead NRA using a 64 μm pore size plankton net, following the U.S. Bureau of Reclamation's protocol (APPENDIX C). The net was lowered to 30 m because a high abundance of veligers are found at that depth (Mueting, 2009). *D. rostriformis bugensis* veliger samples were brought back to the Environmental Health Laboratory at the University of Nevada, Las Vegas where they were subdivided into treatment groups (n = 60 as each replicate in each treatment) and tested in small, glass petri dishes (Glass Petri Dish, 60 x 15 mm; VWR International, Inc.) for analysis. The same collection method, discussed previously, that was used at Lake Mead was also used at Wilson Lake to collect *D. polymorpha* veligers.

Lethal Effects of Quat™ 128 and Quat™ 256 on Dreissenids

Adult *D. rostriformis bugensis* toxicity tests were conducted at the NDOW's hatchery at Lake Mead NRA in a temperature controlled room. Adult *D. polymorpha* toxicity tests were conducted in a laboratory in Wilson, Kansas. Only alive *D. rostriformis bugensis* and *D. polymorpha*, in the fine media mesh bags, were used for experimentation. Four concentrations of Quats™ 128 and 256 were used in the dreissenid adult toxicity tests: control (0), 1, 3, and 5 % solutions. Dreissenids of roughly equal size (N = 192) (>11 mm) were used for the toxicity experiments (12 mussels × 4 treatment groups × 4 replicates = 192 total mussels). Each replicate was placed in a fine media mesh bag and immersed in a 1000 ml beaker with raw Lake Mead water for *D.*

rostriformis bugensis and raw Wilson Lake water for *D. polymorpha* and the appropriate concentration of Quat™ 128 or Quat™ 256 (total volume equaled one liter). The duration of the test was 48 h (Table 3). Because the application of Quat™ 128 and Quat™ 256 will not be used in a reservoir or body of water with fresh lake water, the mussels were not fed nor were they provided air in the beakers. For example, this set up is to mimic a large tank of water that could be filled with lake, tap, or well water for decontaminating wildland firefighting equipment.

Table 3. Experimental design for testing lethal effects of Quat™ 128 and Quat™ 256 on dreissenid adults (n = 48)

Concentration (%)	Exposure Duration (h)					
	6	12	18	24	36	48
0	48	48	48	48	48	48
1	48	48	48	48	48	48
3	48	48	48	48	48	48
5	48	48	48	48	48	48

*Each treatment with four replicates and each replicate with 12 adults

Adult dreissenid mortality was assessed every 6 h up to 48 h using the same protocol discussed previously. In mesh bags, dead *D. rostriformis bugensis* were transferred to a flow through system and *D. polymorpha* were transferred to Wilson Lake and mortality was confirmed 24 h later.

D. rostriformis bugensis and *D. polymorpha* veligers were exposed to four concentrations of Quat™ 128 (0, 0.25, 0.5, and 0.75 %) and Quat™ 256 (0, 0.1, 0.25, and 0.5 %) for the toxicity tests (Tables 4 and 5). The Ecological Effects Test Guidelines for bivalve acute toxicity were followed as outlined by the U.S. EPA (U.S. EPA, 1996). *D. rostriformis bugensis* veliger samples were transported to the Environmental Health

Laboratory at the University of Nevada, Las Vegas in a chilled cooler. Samples were pipetted into a petri dish and examined under a Zeiss DiscoveryV8 stereo microscope (Carl Zeiss,Inc., Peabody, MA) to assess viability. *D. polymorpha* veligers were transported to the laboratory in Wilson, Kansas, in a chilled cooler, where samples were examined under a stereo microscope (Olympus Stereo Zoom, model SZ4045ESD) to assess viability. Both, dead and alive veligers were counted and documented for each petri dish. Veligers that exhibited ciliary movement during a two-minute observation period (Britton & Dingman, 2011), or if internal organs were observed moving, were counted as alive. After the veligers were enumerated, the Quat™ 128 or Quat™ 256 solution was added to the petri dish with a light swirl. Mortality was assessed for all treatment groups at 1, 5, 10, 20, and 40 min and after 40 min for the control groups using cross-polarized light (CPL) (Tables 4 and 5).

Table 4. Experimental design for testing lethal effects of Quat™ 128 on dreissenid veligers

Quat™ 128 (%)	Exposure Duration (min)				
	1	5	10	20	40
0 (control)	60	60	60	60	60
0.25	60	60	60	60	60
0.50	60	60	60	60	60
0.75	60	60	60	60	60

*Each treatment with four replicates and each replicate with 15 veligers

Table 5. Experimental design for testing lethal effects of Quat™ 256 on dreissenid veligers

Quat™ 256 (%)	Exposure Duration (min)				
	1 min	5 min	10 min	20 min	40 min
0 (control)	60	60	60	60	60
0.10	60	60	60	60	60
0.25	60	60	60	60	60
0.50	60	60	60	60	60

*Each treatment with four replicates and each replicate with 15 veligers

Veligers are birefringent because of the crystalline structure of the calcite in the larval shell; hence, they stand out against a dark background. Because of the concentric arrangement of the crystals within the shell, the portions of the shell in line with the axes of the filters are not birefringent thus making the shells appear as glowing crosses (Johnson, 1995). Using CPL microscopy allows for a higher degree of specificity when assessing mortality of veligers. When veligers stop moving, or internal organs appear to stop moving, mortality was assessed. If 100% mortality was not observed within 3 h, the petri dish was set aside and examined every 12 h thereafter, until 24 h was reached.

Effects of Water Temperature on the Efficacy of Quat™ 128 and Quat™ 256

Because the impacts of temperature on the strength of Quat™ 128 and Quat™ 256 may not be significant on adults, only dreissenid veligers were used in this experiment as they are more sensitive to toxicity than dreissenid adults. Before testing the combined variables of water temperature and chemicals, it was important to first, test the efficacy of water temperature and mortality on *D. rostriformis bugensis* and *D. polymorpha* veligers. Previous research shows that *D. rostriformis bugensis* and *D. polymorpha* veligers demonstrate rapid mortality at 30°C and 36°C, respectively (McMahon, 1996). For the current study, veligers were exposed to water temperatures of

2, 16, and 30°C for the durations of 1, 5, 10, 20, and 40 min (Table 6), using heated circular water baths (VWR International Inc.). A shelf was inserted in the bath high enough so that the petri dish was submerged enough without water spilling over the top of the petri dish (Figure 16). The water baths are easily programmed to 16 and 30°C, but cannot be set to freezing levels (i.e., 2°C). However, they can maintain any temperature; hence, ice was added to the water bath to maintain 2°C for the experiments. The water baths have an electronic screen that displays the current temperature for monitoring purposes.

Table 6. Experimental design for testing the effects of water temperature on duration of exposure to Quat™ 128 and Quat™ 256 on killing dreissenid veligers

Water Temperature °C (range)	Exposure Duration (min)				
	1	5	10	20	40
Control	60	60	60	60	60
2 (± 2)	60	60	60	60	60
16 (± 2)	60	60	60	60	60
30 (± 2)	60	60	60	60	60

*Each treatment with four replicates and each replicate with 15 veligers

Figure 16. Dreissenids being exposed to varying water temperatures

After the petri dish with the veliger sample was in the temperature treated water bath for the prescribed time (1, 5, 10, 20, or 40 min), the dish was removed and veliger mortality was assessed under the stereo microscope. Control groups were left on the laboratory bench and did not receive a temperature treated water bath. Mortality of the control groups was assessed after the 40 min treatment time concluded.

Once the data for analyzing the effects of water temperature on dreissenid veliger mortality were collected, the next step was to add the chemical treatment. Concentrations of Quat™ 128 (0, 0.25%, 0.5%, and 0.75%) and Quat ™256 (0, 0.1%, 0.25%, and 0.5%) were added to the petri dish, with veligers before being placed in the water bath. The

above procedure was repeated to assess veliger mortality when exposed to the combined treatments of water temperature and chemical toxicity.

Effects of Ambient Temperature on the Efficacy of Quat™ 128 and Quat™ 256

The toxicity of ammonium compounds is not only associated with concentration and duration of exposure, but is also temperature dependent (Martin et al., 1993). Apart from water temperature, after the solution is exposed to the ambient hot temperature in the field situation, such as summer time in the arid Southwest, the concentration and strength of Quat™ 128 and Quat™ 256 may be reduced as volatilization may occur at higher ambient temperatures. Similarly, some fire incidents experience overnight lows near freezing (e.g., high elevation, northern states, and early or late season fires) that might affect the treatment solution in unknown ways. Thus a wide range of ambient temperatures as represented in fire incidents nationwide were tested.

Only *D. rostriformis bugensis* veligers were used in this experiment as they are a convenient species to sample in the area as opposed to sampling *D. polymorpha* in Wilson Lake, Kansas. Solutions of filtered Lake Mead water and Quat™ 128 (0.25, 0.5, and 0.75 %) and Quat™ 256 (0, 0.1, 0.25, and 0.5 %) were prepared in 500 ml Nalgene bottles and stored at 2, 16, 30, and 43°C for 1, 5, and 10 days in the Emerging Diseases Laboratory at the University of Nevada, Las Vegas (Table 7) using a Frigidaire refrigerator (Model: MRT13CRAZO) for 2°C storage, Wine Enthusiast Silent Touchscreen Wine Refrigerator (Model: 272 03 12) for 15.55°C, and Ultima II Laboratory CO^2 Incubators (REVCO™) for 30°C and 43°C storage. After exposure to the tested ambient temperature, the Nalgene bottles were removed from storage and 100 ml of chemical solution was transferred into a beaker. A 2 ml sample of *D. rostriformis*

bugensis veligers was added to the 100 ml beaker of working solution (total of 102 ml of working solution) (Table 8). The veligers were not pipetted by themselves, as they were in a small water sample. A total of 10 ml (composed of veligers and the working solution) is sufficient enough without being too cumbersome to assess mortality under a microscope in a small petri dish. To get 10 ml sample from the 102 ml beaker, a 50 µm sieve was used to remove 92 ml of solution out of the beaker and the remainder 10 ml was transferred to a petri dish to assess mortality at 5, 10, 20, and 40 min.

Table 7. Effects of ambient temperature on mortality of veligers

Ambient Temperature (°C)	Day 1-Solution	Day 5-Solution	Day 10-Solution
2 (± 2)	60	60	60
16 (± 2)	60	60	60
30 (± 2)	60	60	60
43(± 2)	60	60	60

* Each treatment with four replicates and each replicate with 15 veligers

Table 8. Final working solutions of Quat™ 128 and Quat™ 256 with added veliger sample

Original Concentration Quat™ 128	Final concentration Quat™ 128	Original Concentration Quat™ 256	Final concentration Quat™ 128
.25%	.255%	.1%	.102%
.50%	.51%	.25%	.255%
.75%	.765%	.50%	.51%

Statistical Analysis

ANCOVA was used to test the efficacy of Quat™ 128 and Quat™ 256 on killing *D. rostriformis bugensis* and *D. polymorpha* adults and veligers at different concentrations and durations. ANCOVA was used to test the effectiveness of Quat™ 128 and Quat™ 256 in killing *D. rostriformis bugensis* veligers exposed to differing ambient temperatures. The significance criterion was set at alpha = 0.05. All the statistical analyses were performed using SAS® (Version 9.3, SAS Institute Inc.).

CHAPTER 4

FINDINGS OF THE STUDY

Analysis of Data

Pressurized Water Spray to Remove Dreissenids on Watercraft

A general linear model was used to test if the amount of time a watercraft was out of the water (0, 1, 2, or 4 weeks) would have an effect on time to remove dreissenids. Statistical results showed that the time a watercraft is out of the water, the density, and pressure are significant contributing factors in removing mussels. There is an interaction between the time the watercraft has been out of the water and density as well (ANCOVA, $F_{4, 115} = 13.30$, $p < 0.001$). The time the watercraft is out of the water was the most significant factor affecting removal time ($F_1 = 36.24$, $p < 0.0001$). When the watercraft was just out of the water (week 0), the time to remove *D. rostriformis bugensis* and *D. polymorpha* from watercraft was shorter when mussel density was low with ~1,754 mussels/m^2 (SD = 1,003) and the pressure was set to 3000 psi of water (high) (Table 9). In winter or summer seasons, it was easier to remove mussels from the watercraft when it had been out of the water for at least two weeks or one week, respectively, when compared to being at week 0, or fresh out of the water. The time to remove mussels from watercraft was shorter when mussel density was low and the pressure was high (Table 9). The results showed that it took more time to remove mussels in winter than summer (ANOVA, $F_{43, 44} = p < 0.0001$).

At week 0, it took an average of 430 s to remove a cluster of 11,246 mussels/m^2 (SD = 1,152*)* (high density) of *D. rostriformis bugensis* with 1500 psi (low pressure) in the summer season at Lake Mead, and it took an average of 472 s to remove a cluster of

8,091 mussels/m^2 (SD = 327) (high density) of *D. polymorpha* with 1500 psi of water in the summer season at Wilson Lake (Table 9). Conversely, when 3000 psi of water were used in the summer season on a high density group of *D. rostriformis bugensis*, the time was greatly reduced to 48 s and 52 s for *D. polymorpha* at the two bodies of water, respectively (Table 9). To remove *D. rostriformis bugensis* from a watercraft in the winter season, using high or low psi, it took on average 297 s (range = 202-390 s). Decontamination of *D. rostriformis bugensis* took longer when the watercraft was pulled fresh from the water body in the winter season. It took an average of 346 s (5.77 min) to remove 15,615 m^2 of *D. rostriformis bugensis* from a watercraft. Comparing it to the data from watercraft being out of the water for 2 and 4 weeks, decontamination times were reduced to 3.33 s to remove 21,084 mussels/m^2 and 1.83 s to remove 12,540 mussels/m^2, respectively (Tables 10 & 11).

In the summer season, *D. rostriformis bugensis* and *D. polymorpha* were removed from watercraft using high pressure spray in less time because the byssal threads have a higher chance of drying out faster. However, the longer the vessel is out of the water, the quicker, the mussels will be removed. For instance, to remove *D. rostriformis bugensis* and *D. polymorpha* from watercraft on week 0, using the recommended 3000 psi of water, it took on average 48 s to remove 13,350 mussels/m^2 and 52 s to remove 8,091 mussels/m^2, respectively (Table 9). When the watercraft sat out of the water for 1 week prior to decontamination, the time to remove *D. rostriformis bugensis* and *D. polymorpha* was greatly reduced to 3.5 s to remove 15,776 mussels/m^2 and 4.5 s to remove 7,767 mussels/m^2, respectively (Table 12). Kappel (2012) found that after just 4 hours of exposure to 30ºC ambient temperature, *D. rostriformis bugensis* achieved 30% mortality

which agreed with the data suggesting the *D. rostriformis bugensis* has a lower acute thermal tolerance than *D. polymorpha*, thus explaining the ease in removing the mussels (Spidle et al., 1995; Mills et al., 1996).

There are no data for removal times of *D. polymorpha* in the winter season. However, based on these results with *D. rostriformis bugensis* and *D. polymorpha* having similar removal times in the summer, the data suggest the results from removing *D. rostriformis bugensis* from watercraft, in the winter season, may be applicable to removal times of *D. polymorpha* from watercraft in the winter season. There was no significant difference between *D. rostriformis bugensis* and *D. polymorpha* in removal times (Student-Newman- Keuls multiple comparison, P = 0.81). This suggestion would need to be field validated.

Table 9. Time to remove high or low densities of *D. rostriformis bugensis* and *D. polymorpha* from watercraft in summer and winter seasons using 1500 or 3000 psi of water on week 0

Species	Season	Pressure (psi)	Density (m^2 ± SD)	Time (s)
D. rostriformis bugensis	Summer	1500	High (11,246 ± 1,152)	430 ± 370
D. rostriformis bugensis	Summer	1500	Low (1,909 ± 312)	37 ± 15
D. rostriformis bugensis	Summer	3000	High (13,350 ± 1,136)	48 ± 14
D. rostriformis bugensis	Summer	3000	Low (3,317 ± 335)	43 ± 87
D. rostriformis bugensis	Winter	1500	High (16,586 ± 5,509)	390 ± 140
D. rostriformis bugensis	Winter	1500	Low (1,326 ± 224)	249 ± 174
D. rostriformis bugensis	Winter	3000	High (15,615 ± 258)	346 ± 155
D. rostriformis bugensis	Winter	3000	Low (1,068 ± 1,091)	202 ± 52
D. polymorpha	Summer	1500	High (6,068 ± 530)	472 ± 178
D. polymorpha	Summer	1500	Low (1,262 ± 220)	41 ± 14
D. polymorpha	Summer	3000	High (8,091 ± 327)	52 ± 24
D. polymorpha	Summer	3000	Low (1,246 ± 1,434)	32 ± 32

Table 10. Time to remove high or low densities of *D. rostriformis bugensis* from watercraft in the winter seasons using 1500 or 3000 psi of water on week 2

Pressure (psi)	Density (m^2 ± SD)	Time (s)
1500	High (19,660 ± 8,395)	39.5 ± 15.44
1500	Low (10,784 ± 10,869)	2.33 ± 1.37
3000	High (21,084 ± 4,740)	3.33 ± 1.51
3000	Low (1,553 ± 122.8)	15.5 ± 5.05

Table 11. Time to remove high or low densities of *D. rostriformis bugensis* from watercraft in the winter seasons using 1500 or 3000 psi of water on week 4

Pressure (psi)	Density (m^2 ± SD)	Time (s)
1500	High (12,459 ± 7,256)	18.83 ± 9.32
1500	Low (1,504 ± 419.8)	1.17 ± 0.41
3000	High (12,540 ± 5,820)	1.83 ± 0.98
3000	Low (1,537 ± 444.5)	1 ± 0

Table 12. Time to remove high or low densities of *D. rostriformis bugensis* and *D. polymorpha* from watercraft in the summer season using 1500 or 3000 psi of water on week 1

Species	Season	Pressure (psi)	Density (m^2 ± SD)	Time (s)
D. rostriformis bugensis	Summer	1500	High (12,216 ± 4,760)	8.0 ± 2.3
D. rostriformis bugensis	Summer	1500	Low (1,779 ± 396.3)	15.8 ± 13
D. rostriformis bugensis	Summer	3000	High (15,776 ± 2,972)	3.5 ± 3.8
D. rostriformis bugensis	Summer	3000	Low (1,844 ± 363.3)	8.67 ± 5.2
D. polymorpha	Summer	1500	High (8,576 ± 3,316)	15.6 ± 11
D. polymorpha	Summer	1500	Low (1,375 ± 365)	12.3 ± 15
D. polymorpha	Summer	3000	High (7,767 ± 1,816)	4.5 ± 1.8
D. polymorpha	Summer	3000	Low (1,165 ± 237.8)	6.33 ± 3.8

The data show there was not a significant difference between *D. rostriformis bugensis* and *D. polymorpha* when using pressurized water spray to remove them from watercraft in the summer season (ANOVA, $F_1 = 0.03$, P = 0.81). However, the pressure applied (ANOVA, $F_1 = 25.27$, $p < 0.0001$) and the density of mussels was significant (ANOVA, $F_1 = 26.13$, $p < 0.0001$).

Depending on the size of the watercraft and the amount of biofouling present, decontamination using high pressure spray will take a considerable amount of time. It is

recommended to use 3000 psi of water on the hull, centerboard box and keel (sailboats), lower unit, cavitation plate, and prop. These external areas can handle 3000 psi of water on most watercraft without causing damage and usually have the most amount of mussel fouling. For internal and other sensitive areas of watercraft, manual removal, using brushes and scrapers, of mussels may be necessary. For personal safety, only trained personnel should use high pressure water spray to remove dreissenids from watercraft. If the vessel is left out of the water for at least one week in the summer, or two to four weeks in the winter, the decontamination time can be significantly reduced.

Susceptibility of Zebra Mussels to Hot-water Spray

There was a trend which showed that as the treatment temperatures increased, greater mortality in *D. polymorpha* following the same exposure duration also increased (Table 13). At 70°C, *D. polymorpha* reached 100% mortality within 5 s. *Dreissena polymorpha* reached 100% mortality within 10 s at 54 and 60°C and 5 s at 70 and 80°C treatments (Table 13). Spray exposures of 1 s and 2 s were not found to induce 100% mortality at any of the test temperatures. Treatments of 20°C were ineffective, as only 4% mortality was seen when *D. polymorpha* were exposed to treatment for 80 s (Table 13).

Table 13. Mortality rate (%) of *D. polymorpha* under different treatments by day 10

Temp °C/°F	Exposure Duration (s)							
	1	**2**	**5**	**10**	**20**	**40**	**80**	**160**
20/68	0	0	0	0	0	4	4	0
40/104	2	2	4	4	90	100	100	100
50/122	10	24	38	80	96	100	100	100
54/130	52	72	96	100	100	100	100	100
60/140	84	84	96	100	100	100	100	100
70/158	84	96	100	100	100	100	100	100
80/176	84	96	100	100	100	100	100	100

Note: The mortality of control (n = 6) was 3%.

Estimated LD_{50} values for 1 s, 2 s, 5 s, and 10 s indicate that the temperature to kill 50% of *D. polymorpha* was between 49.8°C to 60.3°C (Table 14). The estimated LD_{99} with these exposure durations varied from >102.8°C at 1 s to 51.1°C at 10 s (Table 14).

Table 14. Estimated LD_{50} and LD_{99} values (in bold) and their 95% confidence limit for hot-water spray treatments on *Dreissena polymorpha* at 1 s, 2 s, 5 s, and 10 s application durations (n = 400 for each duration)

Duration (s)	LD_{50}(°C)	LD_{99}(°C)	SM_{100}(°C)*
1	53.6 < **60.3** < 68.5	> 102.8	> 80
2	44.6 < **56.8** < 69.9	> 86.5	> 80
5	50.7 < **51.2** < 51.7	> 55.0	> 60
10	49.6 < **49.8** < 50.0	50.9 < **51.1** < 51.4	54

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

The mussels in the control groups (n = 200) (mean = 16.02 mm, range = 11.01-21.24 mm) remained in the spat bags immersed in Wilson Lake for ten days. The water temperature of the lake averaged 26.79°C ± 1.7 for the duration of the project. The

control groups and the mussels exposed to 20°C spray treatments exhibited high survival rates. APPENDIX D, figure A, shows that the combined four groups of controls exhibited a 97% survival rate with a range from 96%-98%; APPEDIX D, figure B, shows the eight 20°C spray treatment subsample. These samples displayed a mean 98.5% survival rate with a range of 96% to 100% with no apparent correlation to duration of exposure.

Sixty-seven percent of *D. polymorpha* exposed to 40°C survived treatment. Those mussels that were exposed to that treatment for 1 s and 2 s exhibited a 98% survival rate, and those exposed for 5 s exhibited a 96% survival rate, and when exposed for 10 s, the mussels had a 94% survival rate (APPENDIX D, figure C). Mussels exposed to 40°C and 50°C for 1 s exhibited a 98% and 90% survival rate, respectively. Mussels exposed to 54°C and 60°C for 10 s reached 100% mortality (APPENDIX D, figures E & F), and mussels exposed to 70°C and 80°C reached 100% mortality within 5 s (APPENDIX D, figures G & H). The average shell length of mussels in the 56 treatment groups (n = 2,800) was 16.65 mm (range = 11.02-28.06 mm).

Hot-water Spray Watercraft Validation

The hot-water spray field test showed that 100% mortality of *D. polymorpha* was reached using 54ºC for 10 s of exposure time (APPENDIX D, figure E). These results were validated on a zebra mussel encrusted Crestliner pontoon vessel (Figure 17). Immediate mortality was observed in the six treatment groups (N = 72). No mortality was observed in the six control groups three days post-experimentation (N = 46). The average shell length of the treatment and control group mussels was 8.16 mm (range = 4.84—14.26 mm) and 8.15 mm (range = 6.17—14.15 mm), respectively.

Figure 17. Encrusted pontoon boat from Wilson Lake, Kansas

The results of this study found that *D. polymorpha* are susceptible to hot-water spray. To reach 100% mortality, 54°C for 10 s should be used. The data found in this project and the project looking at high pressure spray can be used in conjunction. To fully kill and remove *D. polymorpha* from watercraft, reducing the biological risk, a combination of high pressure and hot-water spray should be used.

The Efficacy of Quaternary Ammonium Compounds on Killing Dreissenids

Lethal Effects of Quat™ 128 and Quat™ 256 on Dreissenids

ANCOVA showed that the concentrations of Quat™ 128 and Quat™ 256 significantly affected the mortality rate of adult dreissenids with time as a significant covariant (F_{79} = 185.2, $p < 0.0001$). Higher concentrations of Quats™ 128 and 256 resulted in lower numbers of mussel survival and the increased time led to higher numbers of mussel mortality. The time to 100% mortality of adult *D. rostriformis bugensis* decreased with increasing Quat™ 128 and Quat™ 256 concentrations (Figure 18). Similar results were found among *D. polymorpha*: higher concentrations of Quat™ 128 and Quat™ 256 with increased time led to a higher mortality rate (ANCOVA, $p < 0.0001$) (Figure 19). One-way ANOVA showed that the shell lengths of adult *D. rostriformis bugensis* and *D. polymorpha* were not significant in this experiment (*D. rostriformis bugensis*, mean = 18.0 ± 2.49, P = 0.67; *D. polymorpha*, mean = 15.8 ± 312, P = 0.11).

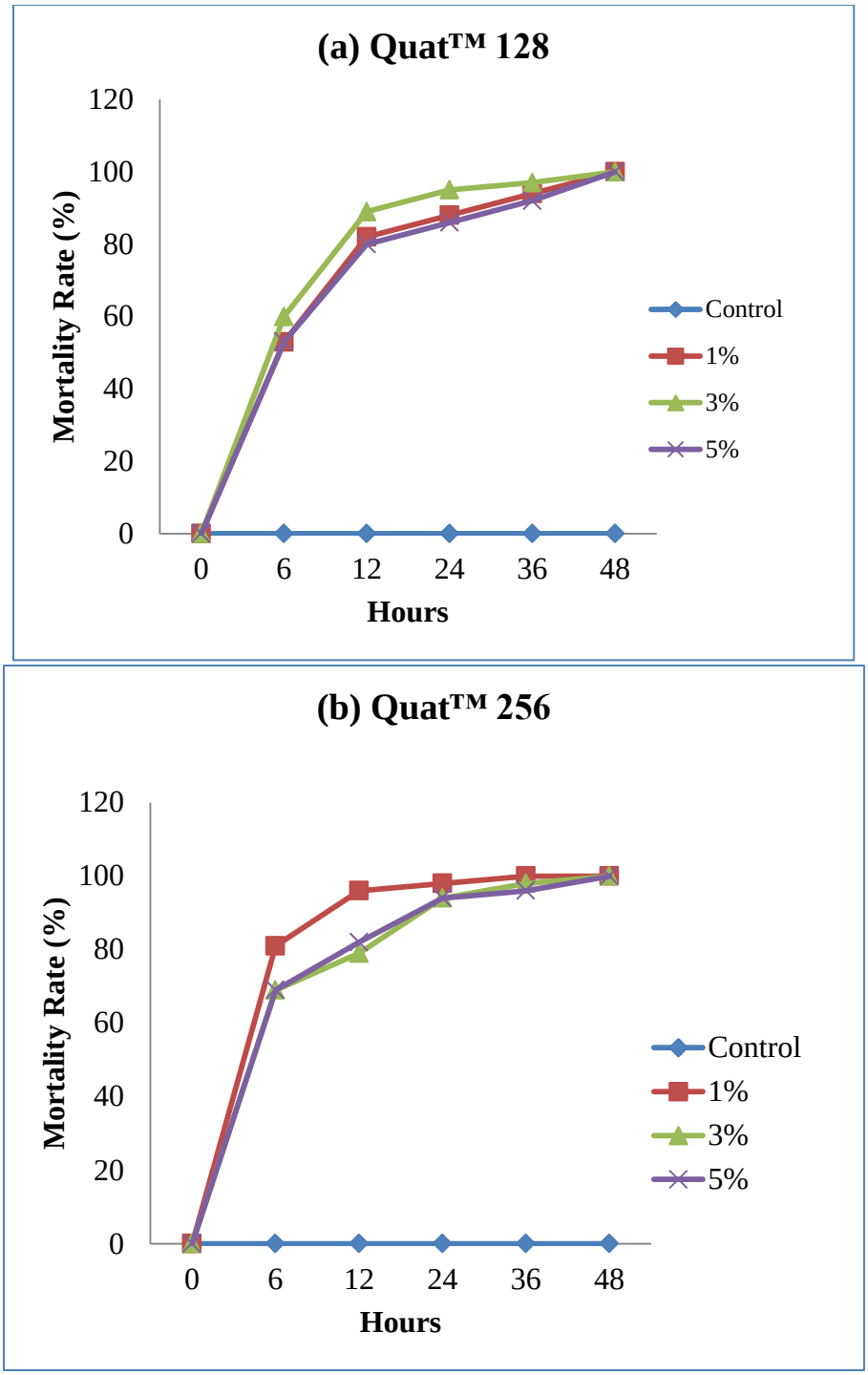
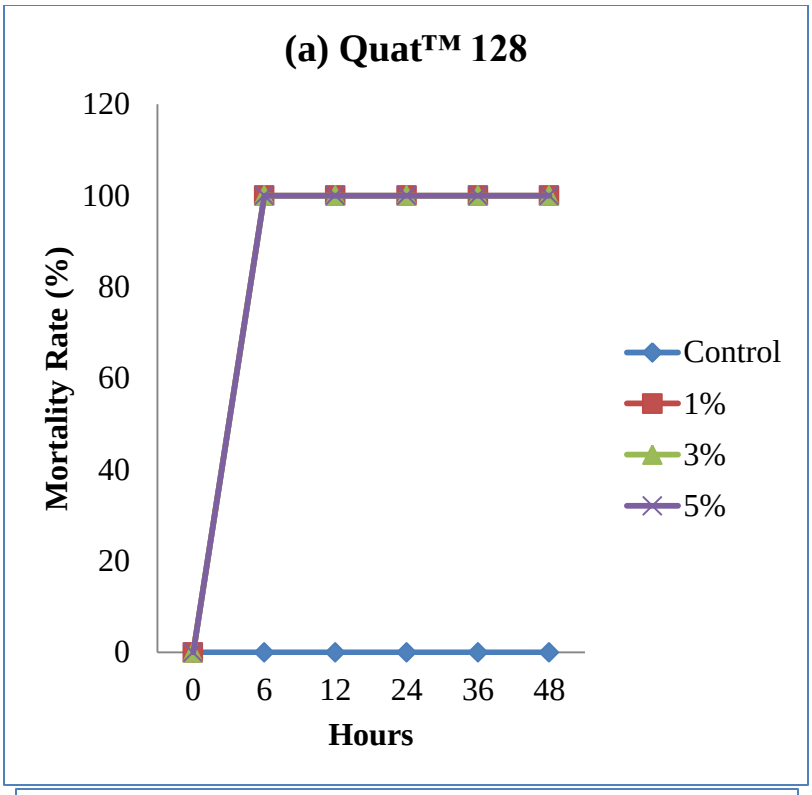


Figure 18. Cumulative mortality of adult *D. rostriformis bugensis* exposed to three concentrations (0, 1, 3, and 5%) of (a) Quat™ 128 and (b) Quat™ 256



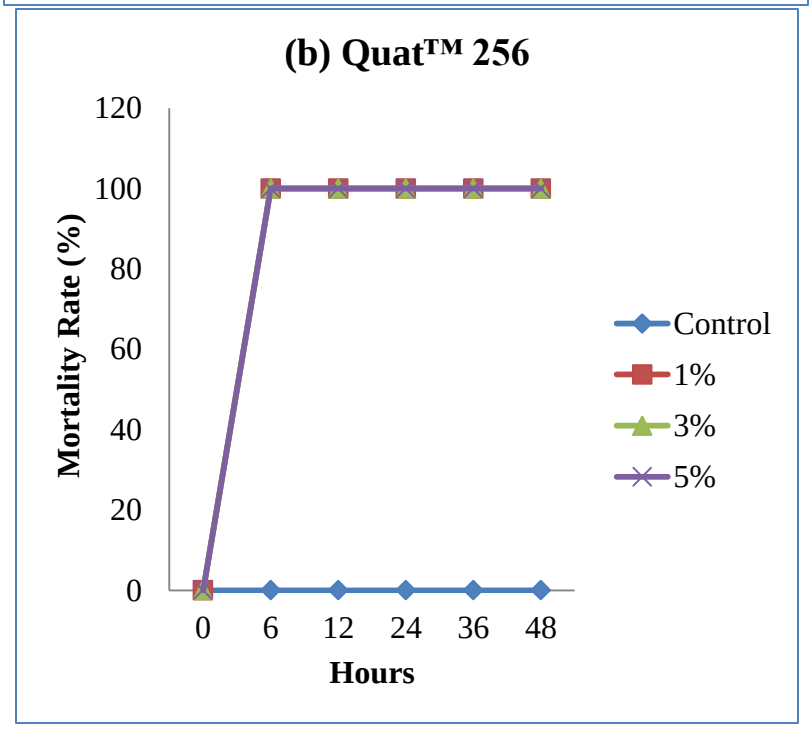


Figure 19. Cumulative mortality of adult *D. polymorpha* exposed to three concentrations (0, 1, 3, and 5%) of (a) Quat™ 128 and (b) Quat™ 256

The mortality rate was greater than 50% within 6 h in the three treatment groups (1, 3, and 5 %) when *D. rostriformis bugensis* were exposed to Quats™ 128 and 256. 100% mortality was reached in all treatment groups by 48 h when *D. rostriformis bugensis* was exposed to Quat™ 128 (Table 15). When *D. rostriformis bugensis* was exposed to Quat™ 256, 100% mortality was reached in all three treatment groups by 36 h (Table 16). Quats™ 128 and 256 induced 100% mortality in *D. polymorpha* within 6 h (Tables 17 & 18). This suggests that adult *D. polymorpha* are more susceptible to Quats™ 128 and 256 compared to *D. rostriformis bugensis*. No mortality occurred in the control groups for adult *D. rostriformis bugensis* and *D. polymorpha* within the 48 h.

Table 15. Mortality rates (%) of *D. rostriformis bugensis* exposed to Quat™ 128 after 48 h (N = 192)

Concentration (%)	**Exposure Duration (h)**					
	0	**6**	**12**	**24**	**36**	**48**
0	0	0	0	0	0	0
1	0	53	82	88	94	100
3	0	60	89	95	97	100
5	0	53	80	86	92	100

Table 16. Mortality rates (%) of *D. rostriformis bugensis* exposed to Quat™ 256 after 48 h (N = 192)

Concentration (%)	**Exposure Duration (h)**					
	0	**6**	**12**	**24**	**36**	**48**
0	0	0	0	0	0	0
1	0	81	96	98	100	100
3	0	69	79	94	100	100
5	0	69	82	94	100	100

Table 17. Mortality rates (%) of *D. polymorpha* exposed to Quat™ 128 after 48 h (N = 192)

Concentration (%)	**Exposure Duration (h)**					
	0	**6**	**12**	**24**	**36**	**48**
0	0	0	0	0	0	0
1	0	100	100	100	100	100
3	0	100	100	100	100	100
5	0	100	100	100	100	100

Table 18. Mortality rates (%) of *D. polymorpha* exposed to Quat™ 256 after 48 h (N = 192)

Concentration (%)	**Exposure Duration (h)**					
	0	**6**	**12**	**24**	**36**	**48**
0	0	0	0	0	0	0
1	0	100	100	100	100	100
3	0	100	100	100	100	100
5	0	100	100	100	100	100

Rapid mortality was observed within minutes when dreissenid veligers were exposed to Quats™ 128 and 256. *D. rostriformis bugensis* reached 100% mortality within 1 min when exposed to 0.5% Quat™ 128 and 0.1% Quat™ 256. *D. polymorpha* reached 100% mortality within 1 min when exposed to 0.5% Quat™ 128 and 0.25% Quat™ 256 (Figures 20 & 21). No mortality was observed of the veligers in the control groups for 40 min.

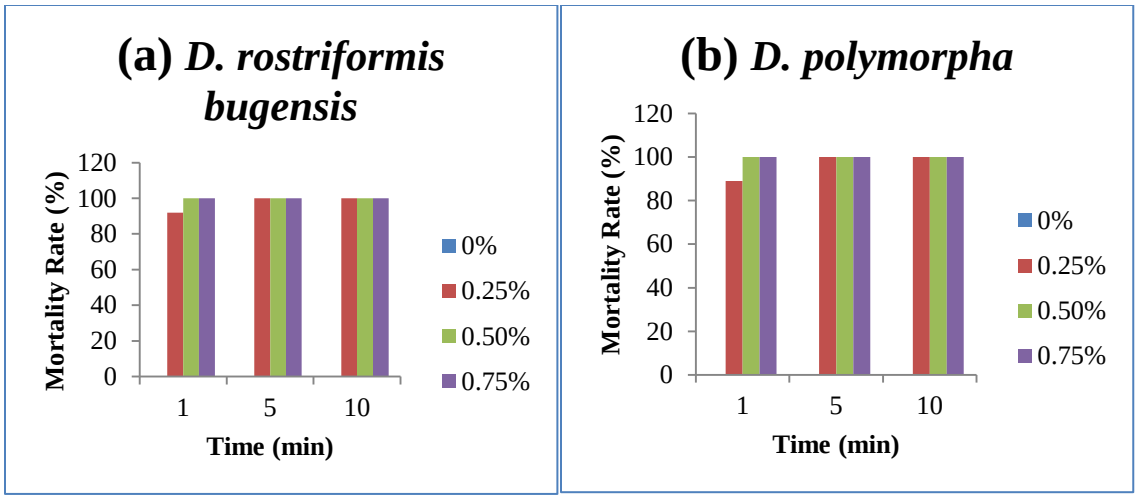


Figure 20. Cumulative mortality of (a) *D. rostriformis bugensis* and (b) *D. polymorpha* veligers exposed to three concentrations (0, 0.25, 0.5, and 0.75%) of Quat™ 128

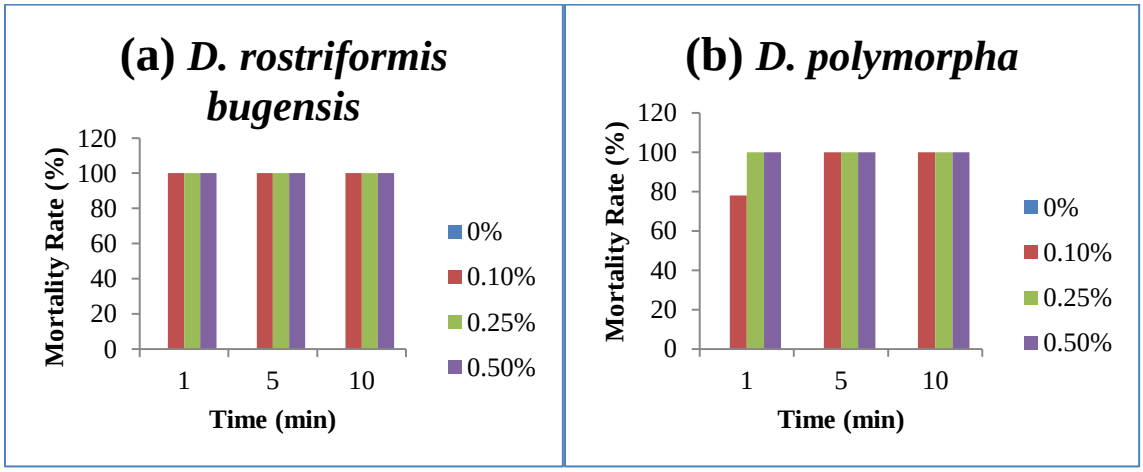


Figure 21. Cumulative mortality of (a) *D. rostriformis bugensis* and (b) *D. polymorpha* veligers exposed to three concentrations (0, 0.1, 0.25, and 0.5%) of Quat™ 256

In a laboratory setting, Quats™ 128 and 256 are effective in killing dreissenid adults and veligers. Adult *D. rostriformis bugensis* reached 100% mortality when exposed to 1% of Quat™ 256 by 36 h. *D. polymorpha* reached mortality at a faster rate compared to *D. rostriformis bugensis*. One percent of Quats™ 128 and 256 are effective

in killing 100% of *D. polymorpha* within 6 h. Dreissenid veligers, being more vulnerable than adults, die within minutes as opposed to hours with less chemical and exposure times. After 1 min of exposure to 0.5% of Quat™ 128, all individual *D. rostriformis bugensis* and *D. polymorpha* died. After 1 min of exposure to 0.1% of Quat™ 256 and 0.25% of Quat™ 256, *D. rostriformis bugensis* and *D. polymorpha* veligers reached 100% mortality, respectively. These results have not been validated in a field setting or on contaminated equipment.

Effects of Water Temperature on the Efficacy of Quat™ 128 and Quat™ 256

Water temperature alone did not induce 100% mortality in *D. polymorpha* veligers, but when *D. rostriformis bugensis* were exposed to 30°C for 20 min, 100% mortality was observed (Figure 22a). The data suggest that *D. rostriformis bugensis* is more sensitive to colder temperatures such as 2°C compared to *D. polymorpha*. *D. rostriformis bugensis* reached a 96% mortality rate after 40 min of exposure, while *D. polymorpha* had a 39% mortality rate at the same time (Figure 22b).

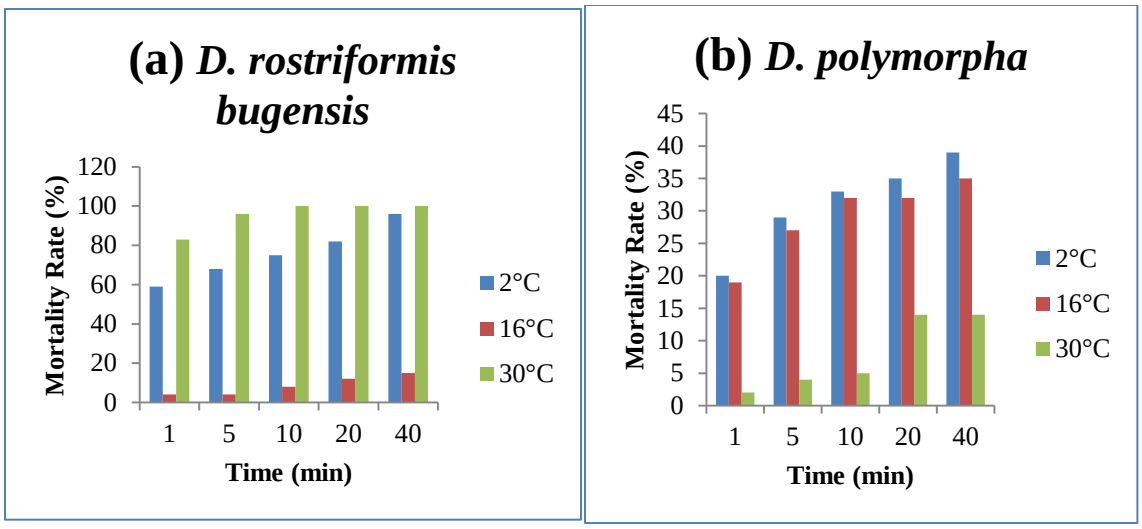


Figure 22. (a) *D. rostriformis bugensis* and (b) *D. polymorpha* exposed to water temperatures of 2, 16, and 30°C (no chemical) for 1, 5, 10, 20, and 40 min

Because mortality rates at all the treatment temperatures were low, ANCOVA was used to determine if the thermal tolerance has an effect on mortality rate. The testing time was short (range = 1-40 min) and the regime was not extreme (ANCOVA, $p > 0.05$). Therefore, there is not a significant difference between *D. rostriformis bugensis* and *D. polymorpha* veligers under the tested thermal conditions.

With the combined variables of water temperature and chemical exposure, rapid veliger mortality was observed. When *D. rostriformis bugensis* veligers were exposed to water temperatures of 2, 16, and 30°C with the combined 0.25, 0.5, and 0.75% Quat™ 128 concentrations for 1 min, 100% mortality was observed in all treatment groups. Eighty-seven percent mortality was observed when veligers were exposed to the 0.25% Quat™ 128 in 2°C water for 1 min of exposure (Figure 23a). *D. rostriformis bugensis* veligers exposed to the same water temperatures coupled with 0.1, 0.25, and 0.5% Quat™ 256 also exhibited a high mortality rate within 1 min. Individuals that were

exposed to 0.1% combined with 2 and 16°C experienced a 78 and 79% mortality rate, respectively (Figure 23b). All other veligers in the treatment groups experienced a100% mortality rate within 1 min. Veligers in the control groups experienced a 100% survival rate.

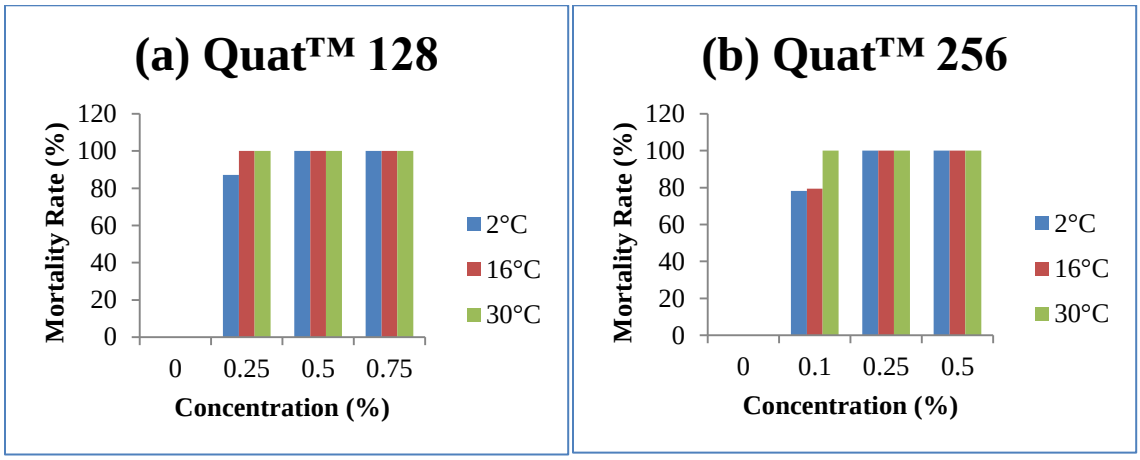


Figure 23. *D. rostriformis bugensis* mortality rate (%) after 1 min exposure to (a) Quat™ 128 and (b) Quat™ 256

D. polymorpha veligers exhibited a lower mortality rate when exposed to both Quats™ 128 and 256 combined with the water temperature treatments of 2, 16, and 30°C compared to *D. rostriformis bugensis*. Only 100% mortality was observed for *D. polymorpha* when individuals were exposed to 0.5 and 0.75% of Quat™ 128 at 2°C and at 0.75% at 16°C for 1 min (Figure 24a). *D. polymorpha* veligers exposed to Quat™ 256 experienced a higher mortality rate compared to those individuals exposed to Quat™ 128. 100% mortality was observed for veligers exposed to 0.50% Quat™ 256 in all water temperature treatments and 0.25% Quat™ 256 in 2°C (Figure 24b).

Because the mortality rate was low for *D. polymorpha* exposed to Quats™ 128 and 256 combined with the water treatments (2, 16, and 30°C) for 1 min, the exposure time was increased to 5 min. 100% mortality was observed in treatment groups exposed to 0.5% and 0.75% Quat™ 128 in 2°C and > 83% mortality was observed the other treatment groups within 5 min (Figure 25a). *D. polymorpha* exposed to 0.25 and 0.5% Quat™ 256 in all water treatment groups exhibited 100% mortality, and veligers exposed to 0.1% Quat™ 256 in all water treatment groups exhibited > 94% mortality within 5 min (Figure 25b). Dreissenid veligers exposed to the combination of water temperature and Quat™ 256 seem to have a higher mortality rate compared to the veligers exposed to the Quat™ 128 solutions. This could be because the active ingredient [blend of DDAC (10.14%) and ADBAC (6.76%)] found in Quat™ 256 is twice as much as the blend found in Quat™ 128. All *D. polymorpha* veligers in the control groups experienced a 100% survival rate.

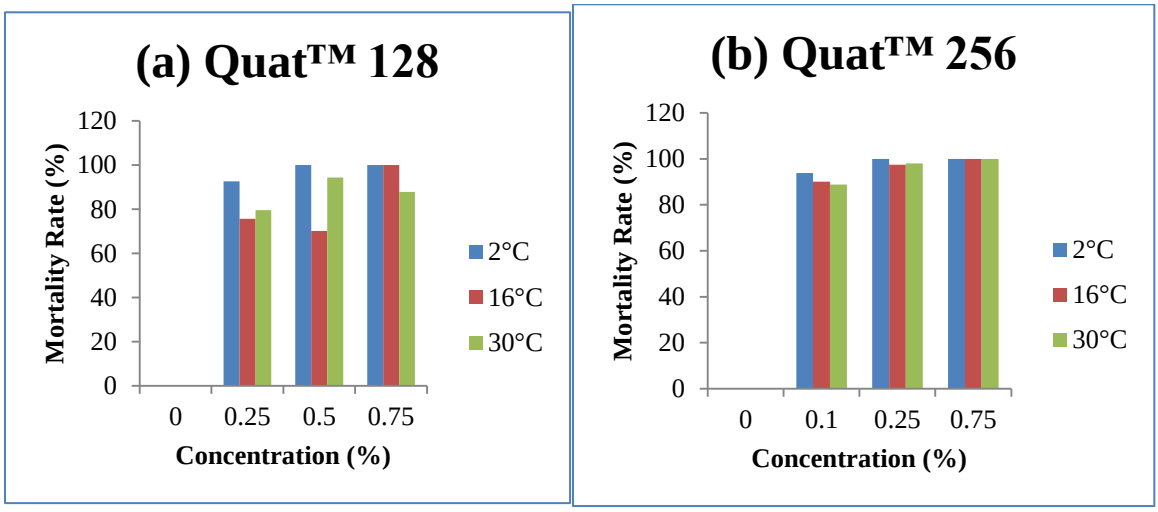


Figure 24. *D. polymorpha* mortality rates (%) after 1 min exposure to (a) Quat™ 128 and (b) Quat™ 256

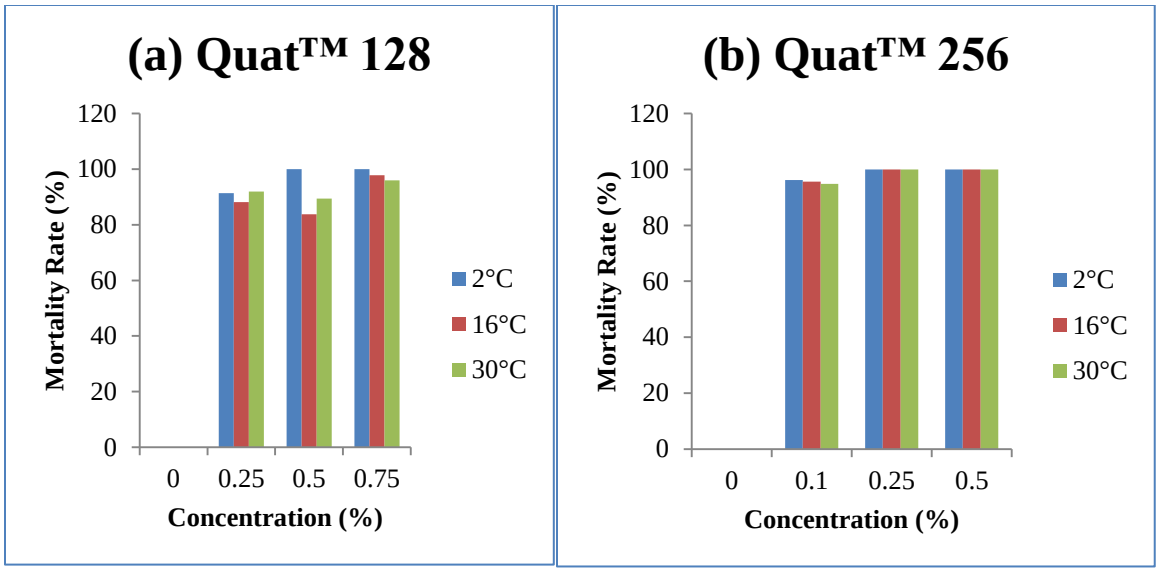


Figure 25. *D. polymorpha* mortality rates (%) after 5 min exposure to (a) Quat™ 128 and (b) Quat™ 256

It is recommended to use 0.5% Quat™ 128 for 1 min or 0.25% Quat™ 256 for 1 min to decontaminate wildland firefighting equipment exposed to *D. rostriformis bugensis*. To decontaminate equipment exposed to *D. polymorpha*, it is recommended to use 0.75% Quat™ 128 for 5 min or 0.25% Quat™ 256 for 5 min. These recommendations are made based on the results that showed a 100% mortality rate in all water treatment groups with the smallest chemical concentration and exposure time. When the species is unknown, use the most conservative recommendation of 0.75% Quat™ 128 for 5 min or 0.25% Quat™ 256 for at least 5 min.

Effects of Ambient Temperature on the Efficacy of Quat™ 128 and Quat™ 256

Quat™ 128

Differing concentrations of Quat™ 128 (0.25, 0.5, and 0.75 %) being stored in the four different ambient temperatures (2, 16, 30, and 43°C) for 1, 5, or 10 days had a significant impact on *D. rostriformis bugensis* veligers (N = 2,783) mortality rate (F_{11},

8996.03, $p < 0.0001$). The length of time at which the chemical concentrations of Quat™ 128 were stored was significant for the model ($F_2 = 3.76$, $p < 0.024$). However, there was no significant difference in the effectiveness of Quat™ 128 between days 5 and 10 and days 5 and 1, but the mortality rate from the 10 day storage is significantly higher than that from the day 1 storage (Student-Newman- Keuls multiple comparison, $p > 0.05$). When examining the effects of ambient temperature on the effectiveness of Quat™ 128, the results showed there was no significant difference between 30 and 43°C, whereas 16°C was the only temperature that was significant (Student-Newman- Keuls multiple comparison, $p > 0.05$). Also, the time to 100% mussel mortality showed no significant difference between 20 and 40 min (Student-Newman- Keuls multiple comparison, $p > 0.05$). Finally, the concentrations of Quat™ 128 (0, 0.25, 0.5, and 0.75%) were all significantly different from one another. Based on these results, storage time should not be a concern for Quat™ 128, and more focus should be placed on the chemical concentration and contact time to fully decontaminate the equipment. However, it is not recommended to store Quat™ 128 solutions beyond 10 days because its effectiveness for killing dreissenid veligers has not been tested.

Quat™ 128 stored or used at the ambient temperature of 2°C, 0.25% and 0.5% induced 100% mortality within in 40 min and 0.75% induced 100% mortality within 20 min (Figure 26 a, b, & c). The results showed that the ambient temperature of 16°C, at all concentrations of Quat™ 128 induced 100% mortality within 20 min and 0.75% induced mortality within 10 min (Figure 27 a, b, and c). At an ambient temperature of 30°C, 100% mortality of *D. rostriformis bugensis* was reached in all Quat™ 128 concentrations within 10 min and 0.75% within 5 min (Figure 28 a, b, & c). Finally,

100% mortality was recorded within 10 min when mussels were exposed to all Quat™ 128 concentrations that were stored at 43°C and 100% mortality was recorded at 5 min when mussels were exposed to 0.75% Quat™ 128 (Figure 29 a, b, & c) (Table 19). Given the factors of chemical toxicity, water temperature and ambient temperature, explored in these projects, it is recommended to use 0.25% Quat™ 128 for 40 min to reach 100% mortality in dreissenid veligers. Out of the control groups (n = 858), 1.3% of the individuals experienced mortality.

Table 19. Minimum time (min) to induce 100% mortality in *D. rostriformis bugensis* using 0.25, 0.5, and 0.75% Quat™ 128 in 2, 16, 30, and 43°C ambient temperatures

Concentration (%)	2°C	16°C	30°C	43°C
0.25	40	20	10	10
0.50	40	20	10	10
0.75	20	10	5	5

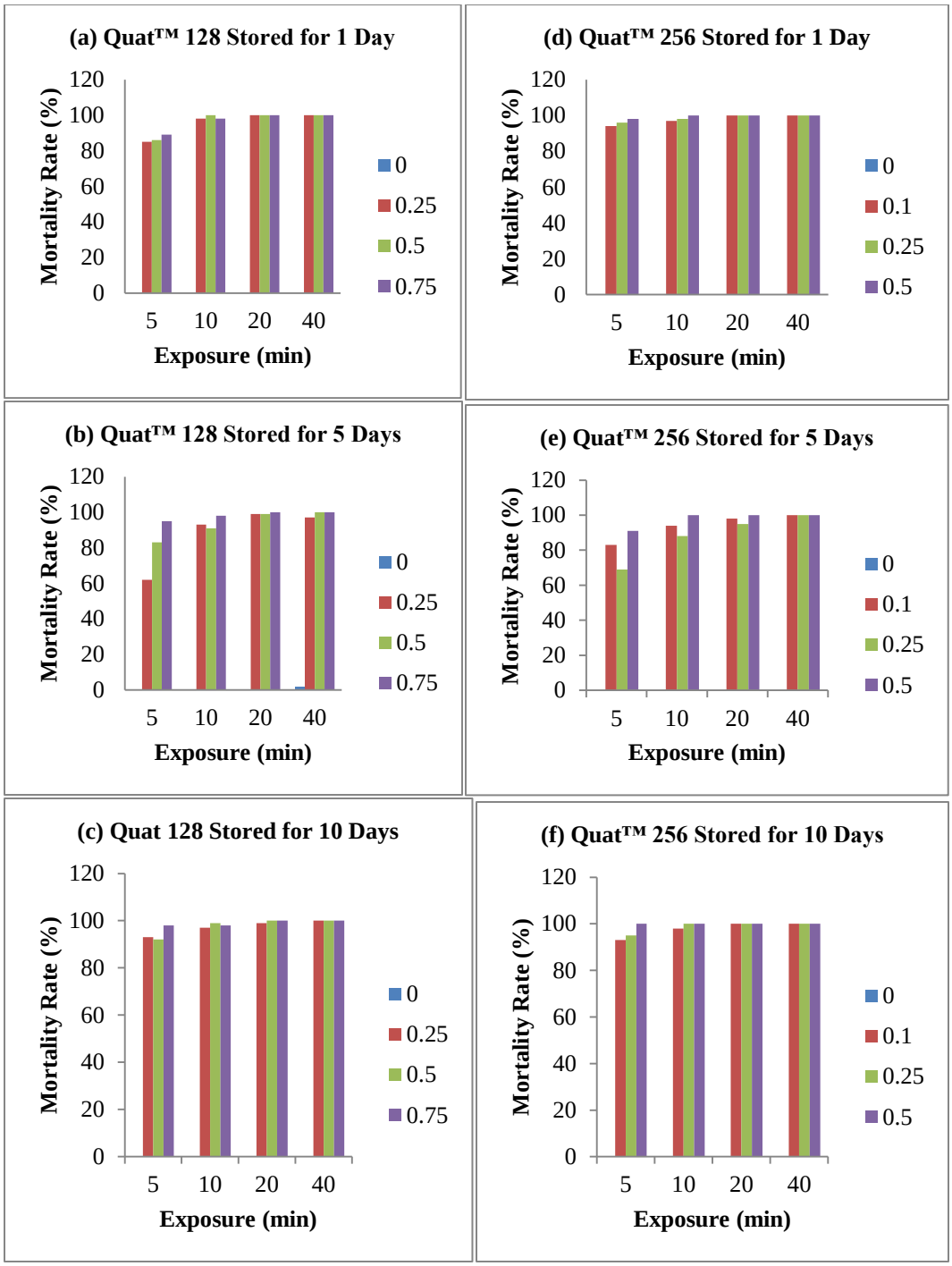


Figure 26. Mortality rate (%) for *D. rostriformis bugensis* exposed to three different Quat™ 128 concentrations that have been stored for (a) 1, (b) 5, and (c) 10 days at 2°C and three different Quat™ 256 concentrations that have been stored for (d) 1, (e) 5, and (f) 10 days at 2°C

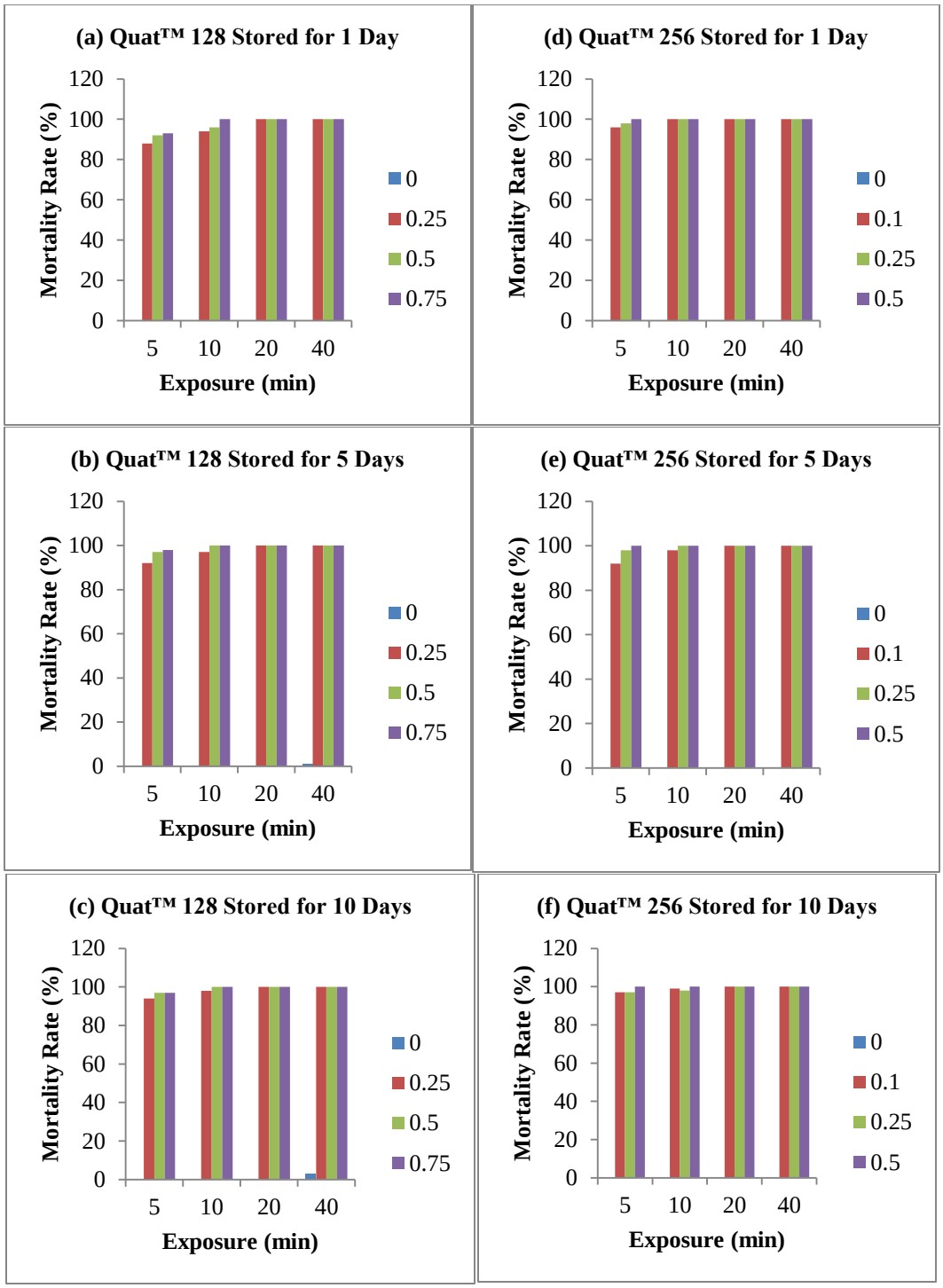


Figure 27. Mortality rate (%) for *D. rostriformis bugensis* exposed to three different Quat™ 128 concentrations that have been stored for (a) 1, (b) 5, and (c) 10 days at 16°C and three different Quat™ 256 concentrations that have been stored for (d) 1, (e) 5, and (f) 10 days at 16°C

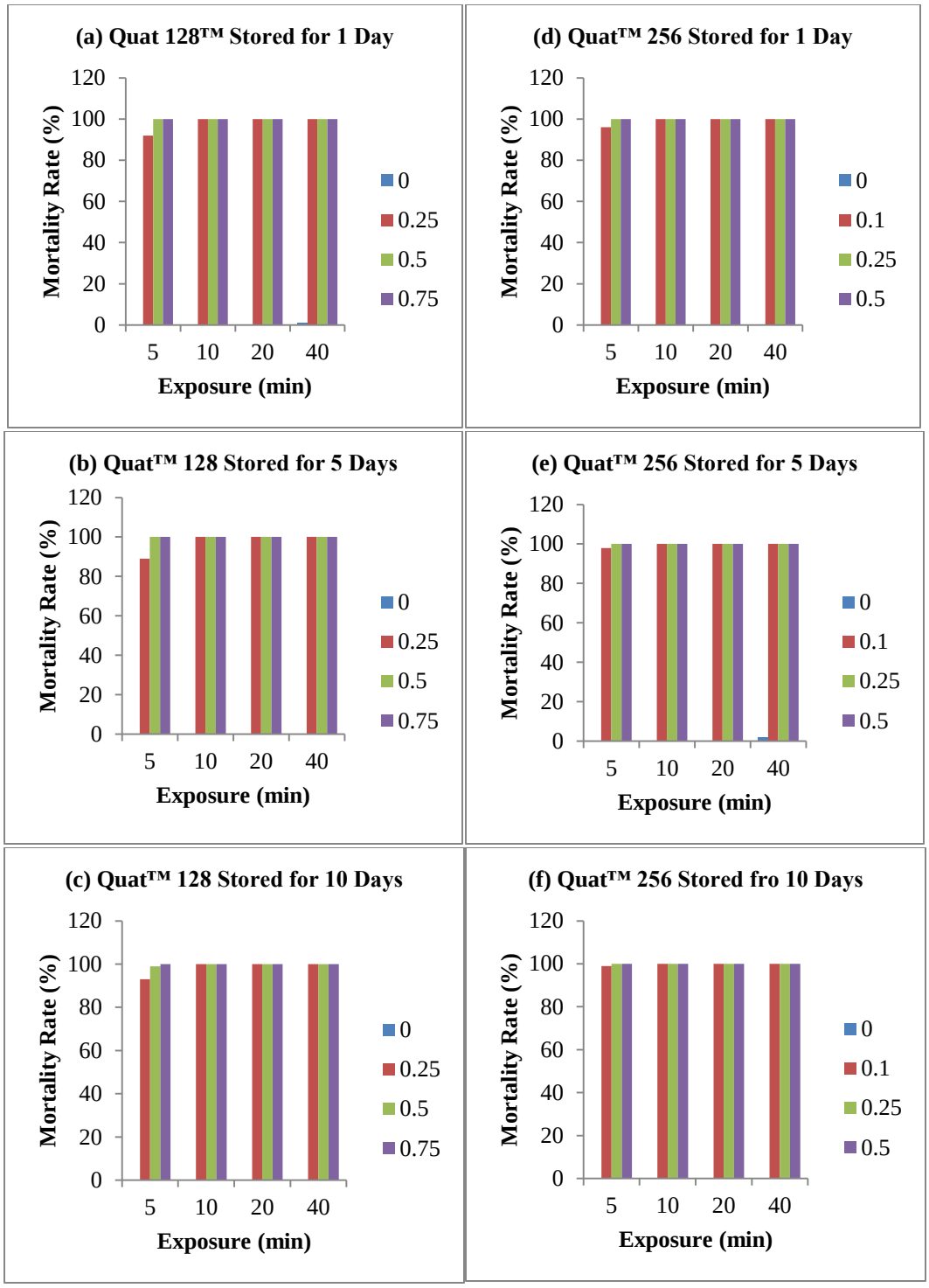


Figure 28. Mortality rate (%) for *D. rostriformis bugensis* exposed to three different Quat™ 128 concentrations that have been stored for (a) 1, (b) 5, and (c) 10 days at 30°C and three different Quat™ 256 concentrations that have been stored for (d) 1, (e) 5, and (f) 10 days at 30°C

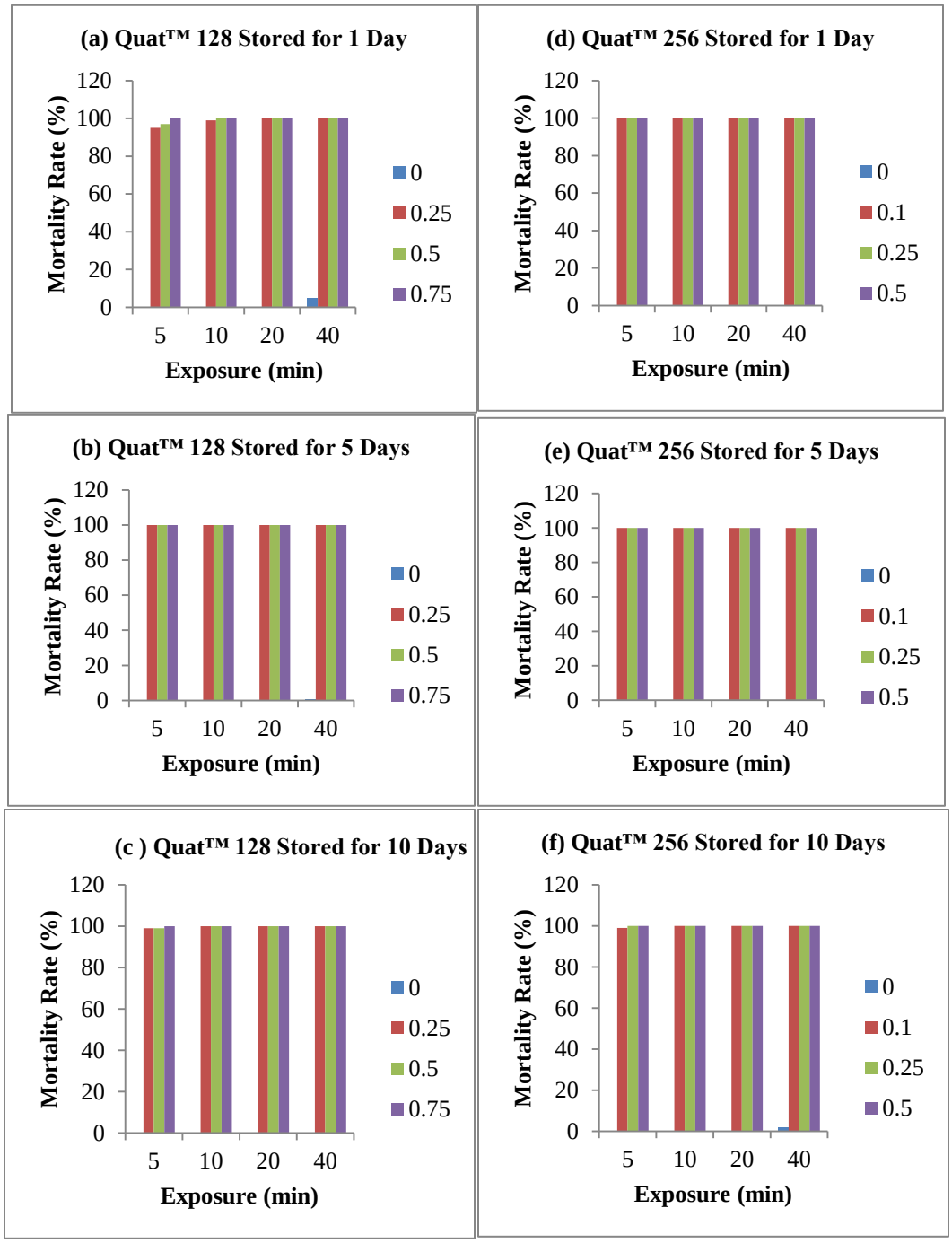


Figure 29. Mortality rate (%) for *D. rostriformis bugensis* exposed to three different Quat™ 128 concentrations that have been stored for (a) 1, (b) 5, and (c) 10 days at 43°C and three different Quat™ 256 concentrations that have been stored for (d) 1, (e) 5, and (f) 10 days at 43°C

Quat™ 256

The results showed that differing concentrations of Quat™ 256 (0.1, 0.25, and 0.5%) being stored in the four different ambient temperatures (2, 16, 30, and 43°C) for 1, 5, or 10 days, are similar to the Quat™ 128 results (Figures 26-29, d, e, and f). Quat™ 256 had a significant impact on *D. rostriformis bugensis* veliger (N = 2,671) mortality rate (F_{11}, 1210.25, $p < 0.0001$). When concentrations of Quat™ 256 were stored for 10 days, mortality rate was significantly lower than that from the day 1 storage, which is the opposite from the results evaluating Quat™ 128. There was no significant difference between storage days 1 and 5 (Student-Newman- Keuls multiple comparison, $p > 0.05$). When evaluating the effects of ambient temperature on veliger mortality rate, the Quat™ 256 results mirrored the findings from Quat™ 128. There was no significant difference between 30 and 43°C (Student-Newman- Keuls multiple comparison, $p > 0.05$). When looking at the time to mussel mortality, 20 min was significantly different from 5, 10, and 40 min (Student-Newman- Keuls multiple comparison, $p < 0.05$). Finally, the results show that unlike the concentrations of Quat™ 128, concentrations of Quat™ 256 are not significantly different among groups, aside from the control group (Student-Newman-Keuls multiple comparison, $p > 0.05$).

When Quat™ 256 was used or stored at 2°C ambient temperature, 0.1 and 0.25% induced 100% mortality within 40 min and within 20 min using 0.5% (Figure 26 d, e, & f). At 16°C ambient temperature, 0.1% induced mortality within 40 min, 0.25% within 10 min, and 5 min induced mortality using 0.5% Quat™ 256 (Figure 27 d, e, & f). At 30°C, 100% mortality of *D. rostriformis bugensis* was reached within 10 min using 0.1% and within 5 min using 0.25% and 0.5% (Figure 28 d, e, & f). Finally, 100% mortality

was recorded within 10 min when mussels were exposed to 0.1% Quat™ 256, and 0.25% and 0.5% for 5 min (Figure 29 d, e, & f) (Table 20). Given the factors of chemical toxicity, water temperature, and ambient temperature, explored in these projects, it is recommended to use 0.1% Quat™ 256 for 40 min to reach 100% mortality in dreissenid veligers. The control groups (n = 850) had a high rate of survival with only 0.9% of the individuals experiencing mortality.

Table 20. Minimum time (min) to induce 100% mortality in *D. rostriformis bugensis* using 0.1, 0.25, and 0.5% Quat™ 256 in 2, 16, 30, and 43°C ambient temperatures

Concentration (%)	2°C	16°C	30°C	43°C
0.1	40	40	10	10
0.25	40	10	5	5
0.5	20	5	5	5

CHAPTER 5

DISCUSSION, CONCLUSIONS, & RECOMMENDATIONS

Discussion of Results

Following the discovery of *D. rostriformis bugensis* in the Lower Colorado River system, numerous decontamination methods have been studied to reduce the further spread of dreissenids. One of the aims of this dissertation was to evaluate the effectiveness of pressurized and hot-water spray in killing and removing dreissenids from watercraft. The overall goal was to recommend safe, quick, and effective protocols to reduce the biological risk of watercraft moving to un-infested bodies of water. The second objective was to evaluate the effectiveness of Quat™ 128 and Quat™ 256 on killing dreissenid adults and veligers with the goal of providing safe, quick, and effective protocols to decontaminate wildland firefighting equipment.

Pressurized Water Spray to Remove Dreissenids on Watercraft

The spread of *D. rostriformis bugensis* and *D. polymorpha*, as well as other AIS, can be attributed to overland movement of watercraft (Johnson et al. 2001; Leung & Bossenbroek, 2006). These vessels can range from small fishing boats, pontoon boats, wakeboard boats to larger watercrafts such as houseboats and yachts. Boat movement, with attached AIS, has been an increasing concern among freshwater management agencies. To combat these concerns, federal, state, and local lake associations have set up entrance and exit inspection and decontamination stations. Depending on the managing agency, these stations are set up along major highways into the state or on the launch ramp of a water-body. The goals of the inspection and decontamination stations are to kill and remove all AIS, especially *D. rostriformis bugensis* and *D. polymorpha*.

Killing prevents establishment of new populations as a result of watercraft transfer, but removing them is also important because a false positive finding may result from the presence of mussel shells and pieces, such as DNA left in the samples. Although they are dead, unnecessary concern and expensive action could happen if unexplained shells drop or are scraped-off the watercraft and subsequently discovered at an inspection station, launch ramp, or found in a lake (Zook & Phillips, 2012).

Since *D. rostriformis bugensis* has invaded the western U.S., boating practices have changed. When a boater exits an infested body of water, they are asked to clean, drain, and dry their vessel prior to leaving. When the vessel has visible mussels attached or they have been on the water for a specified time (usually more than five consecutive days), they are asked to go through an AIS removal station.

Physical removal of dreissenids using scraping, hand picking, and pressurized water spray is the most obvious and labor intensive control method for decontamination (QZAP, 2010). It is easy to achieve less than 100% removal rate of mussels using these strategies, as it is difficult to remove every mussel, thus limiting their usefulness in preventing the spread of dreissenids. Scraping and hand picking mussels may be effective when infested surfaces are flat and there are no hidden places containing mussels that can be overlooked.

The objective of this project was to evaluate the quickest and most effective way to decontaminate a vessel by removing dreissenids using high pressure water spray. The results of the study showed that 3000 psi of water is superior to 1500 psi of water, as it is strong enough to remove the mussels without damaging the vessel. However, when decontaminating certain areas on the vessel, such as the gimbal unit, it is recommended to

avoid using pressurized water spray as it can damage some of the seals and parts in that area (Zook & Phillips, 2012). If the equipment is capable, 3000 psi of water should be used when removing dreissenids from watercraft in all seasons.

The results also indicated that *D. rostriformis bugensis* and *D. polymorpha* are removed from a vessel at a faster rate when it has been out of the water for at least one week in the summer and two weeks in winter time. In winter time, temperatures are lower and humidity is generally higher compared to summer time when temperatures are dry and hot. When a vessel is fresh out of the water (week 0), it is more difficult and takes much longer to remove dreissenids because the byssal threads have not had a chance to dry out and weaken. When *D. rostriformis bugensis* were exposed to ambient temperatures between 20 and 40°C, they did not survive for more than one day, regardless of relative humidity (Kappel, 2012). Mussels exposed to 10°C ambient temperatures reached mortality after 5 days. As mussels die, their byssal threads dry out and weaken, hence reducing their function. Compared with *D. rostriformis bugensis*, *D. polymorpha* has a significantly higher byssal thread synthesis rate, lower dislodgment in flow, and requires greater force for mechanical detachment (Peyer, McCarthy, & Lee, 2009). While 3000 psi of water is the recommended protocol for removing dreissenids from watercraft, 1500 psi of water may be useful when the mussels are dead and the byssal threads have dried out. It would not take as much effort to remove these mussels. However, field tests would need to be conducted before a recommendation can be made.

To save time and to be less of an inconvenience to boaters, if hot water is unavailable for AIS removal, (see more from hot-water spray below), it is suggested that the vessel remain out of the water for at least one week in the summer and two weeks in

the winter before the high pressure spray decontamination method is used. However, this may be difficult to achieve at some water bodies, as there may not be places to store the vessel prior to decontamination. However, using 3000 psi of water on a watercraft fresh out of the water will suffice for removing attached mussels.

Susceptibility of Zebra Mussels to Hot-water Spray

When decontaminating watercraft, it is important not only to remove the mussels, but also ensure they are dead. When removing the mussels with high pressure spray, it is difficult to remove every mussel as some cannot be reached or they can be dislodged, landing on a different spot or on the trailer; however, using hot-water spray will certify that the mussels are at least dead. Hot-water spray is sustainable, effective, and economical compared to chemical applications which could lead to further financial and ecological issues (Piola, Dafforn, & Johnston, 2009). By using tap-water cultured *D. polymorpha*, Morse (2009) found that water sprayed at ≥ 60°C for 10 s or 80°C for ≥ 5 s was 100% lethal to *D. polymorpha*, which indicates that current decontamination recommendations of spray temperature of ≥ 60°C may not kill all the mussels if the exposure duration is < 10 s. The current study found the same results as Morse (2009) in regards to 10 s application using 60°C will result in 100% *D. polymorpha* mortality. The results also showed that with a 5 s application, using 60°C resulted in a 98% mortality rate, as opposed to Morse's study that concluded 5 s application resulted in an 87% mortality rate. However, at a cooler temperature of 54°C, 100% mortality of *D. polymorpha* was also attained by 10 s in this study. The experimental design was set up to examine lethal temperatures at 5 and 10 s, whereas 6, 7, 8, and 9 s was not tested. Through the results of this study, it is suggested that *D. polymorpha* could reach 100%

mortality between 6-9 s when exposed to 60°C. In that case, boat inspectors can save time in decontaminating a mussel fouled watercraft. Field tests would be needed to validate this assumption.

In Morse's (2009) study, the LT_{50} and LT_{99} at 1 s duration were both >80°C while they were 60.3°C and >102.8°C, respectively for *D. polymorpha* in the present study (Table 14). At 5 s duration, LT_{50} and LT_{99} for *D. polymorpha* in the first study were 54.6°C and 69.1°C while they were 51.2°C and > 55.0°C in the current study. With 10 s exposure, the LT_{50} and LT_{99} for *D. polymorpha* in the first study were 46.9°C and 53.9°C, and in the current study, they were 49.8°C and 51.1°C. Clearly, relatively lower temperature with the same exposure time, or relatively less time under the same treatment temperature is needed to reach the same lethal rate in the present study than the study by Morse (2009). The only difference is that *D. polymorpha* in Morse's study have been acclimated in laboratory conditions while the present study used mussels fresh from the native reservoir. Therefore, the physiology of *D. polymorpha* tested in these two studies and their responses may differ (Costa, Aldridge, & Moggridge, 2008). The effect of adaptation of physiological responses of *D. polymorpha* to hot-water treatment needs to be studied in the future.

Another study examined the susceptibility of *D. rostriformis bugensis* to hot-water sprays. The researchers found that at hot-water temperatures ≥60°C, with contact duration of only 5 s was sufficient to induce 100% mortality in *D. rostriformis bugensis* (Comeau et al., 2011). These results indicate that *D. rostriformis bugensis* are more susceptible to hot-water sprays than *D. polymorpha* when comparing to the results from the current study and Morse's study. *D. rostriformis bugensis* have thinner shells

(Zhulicov et al., 2006) and less tightly sealing shell valves compared to *D. polymorpha* (Claxton et al., 1997). Because the shell valves may not close as tightly in *D. rostriformis bugensis*, heating of the soft tissues may occur more rapidly than that of *D. polymorpha*. 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 & Klerks, 2005). The upper thermal limit of *D. rostriformis bugensis* is lower than that of *D. polymorpha* (McMahon, 1996). This suggests that *D. rostriformis bugensis* are more susceptible to death by hot-water sprays at a lower temperature and less exposure time than *D. polymorpha*.

The results from hot-water spray watercraft validation test were not surprising. Once the laboratory test was completed and the temperature and time needed to reach 100% mortality in *D. polymorpha* was determined, the field validation was conducted. The field tests exposing *D. polymorpha* to 54°C for 10 s, verified the laboratory tests and 100% mortality was observed immediately after treatment.

There are a couple of other methods for watercraft decontamination that have been explored, such as dry time acceleration and dry ice blasting (Zook & Phillips, 2012). The rate of desiccation for dreissenid mussels is a function of temperature, humidity, and mussel size (Morse, 2009). Increasing ambient temperatures and lower humidity decrease the time needed for desiccation, while larger mussels require more time to dry-out than smaller mussels. To assist lake managers in knowing how long a watercraft needs to remain out of the water to ensure the mussels are dead through desiccation, a dry time estimator was developed (100th Meridian Initiative, 2011). The estimator is a tool

that can be used to estimate the minimum time a vessel should remain out of the water before launching in an uninfested water body. Accelerated dry times should be used after a fouled vessel has gone through a high pressure, hot-water spray service.

The use of dry ice (CO_2) pellets for cleaning and removing attached dreissenids is an alternative decontamination method. Dry ice blasting uses compressed air to propel tiny dry ice pellets onto fouled watercraft. The dry ice freezes the mussels and kills them. The pellets quickly dissipate into the air so there is no wastewater or other media to dispose (Zook & Phillips, 2012). This will only remove the mussels, not kill them. For high density colonization, 100% mortality rate could not be reached for removed mussels (WH Wong, personal communication). The effectiveness of dry ice blasting has not been reviewed in the literature and it should be systematically investigated prior to implementation. Combining the methods of pressurized (3000 psi of water) and hot-water spray (60°C for 10 s) to the surface of the fouled watercraft is the best way to decontaminate a vessel to prevent the further spread of dreissenids. For the inaccessible areas, such as the gimbal area, inside the engine, generator and AC cooling systems, treatments of 60°C for 10 s will not suffice. According to Comeau et al. (2011), the amount of time needed to achieve the target lethal temperature is 43 s for the summer time, and 2 minutes and 7 s for the winter time. The time variations are because of the different surface area temperatures present between the two seasons. The hot water needs to warm up these internal compartments. In addition, most watercraft have special areas that have water transfer pumps that require water temperature ≤ 49°C for decontamination, such as ballast tanks/bladders, wash-down systems, bait and live wells, and internal water systems. For these sensitive areas, it is recommended that the

temperature of the hot-water flush be monitored until a temperature of 49°C is reached. After this target temperature is reached, it is necessary to maintain a constant flush of that temperature for at least 10 s to ensure 100% mussel mortality (Comeau et al., 2011).

Again, it is extremely difficult to remove every single mussel from an infested watercraft. Combining the methods ensures that the decontamination process is removing mussels and killing them; hence reducing the biological risk.

The Efficacy of Quaternary Ammonium Compounds on Killing Dreissenids

When using pressurized and hot-water sprays as a form of decontamination is not feasible, other methods, such as chemical control can be used. The most popular and least expensive chemical used for control of aquatic invasive mussels is chlorination (Claudi & Mackie, 1994; Rajagopal et al., 1996; Sprecher & Getsinger, 2000). The benefits of chlorine are that it is effective at low concentrations and efficient against all fouling categories ranging from bacteria to mollusks. It not only kills adult dreissenids, but is effective in preventing veligers from settling on pipes and other substrates (Jenner & Janssen-Mommen, 1993). However, chlorine is not a viable candidate for decontaminating wildland firefighting equipment as it can be corrosive over time, causing damage to some of the components, such as fabric, metals, and gaskets (U.S. FS, 2012). Many ammonium compounds have been registered for use in *D. polymorpha* control, such as BULB6002, Calgon H-130M, and ClamTrol (Sprecher & Getsinger, 2000); however, no systematic study on the efficacy of Quat™ 128 and Quat™ 256 on killing dreissenid adults and veligers has been conducted.

The U.S. FS's invasive species program was created to reduce, minimize, or eliminate the potential for introduction, establishment, spread, and impact of invasive

species across all landscapes. Wildland firefighting equipment is a secondary mode of transport in moving dreissenids. To combat other AIS, such as *Myxobolus cerebralis* (causative agent of whirling disease), New Zealand mudsnail, chytrid fungus, didymo, and dreissenid mussels, various concentrations of quaternary ammonium compounds (e.g., blends of ADBAC and DDAC) are prescribed (U.S. FS, 2012). Firefighter and public safety is still the first priority; however, AIS pose a risk to both the environment and to firefighting equipment. Once veligers settle and grow, they can clog valves and pumps if equipment is not completely drained or treated. It is crucial that firefighting equipment remains operational and with avoidance or decontamination protocols, this can be attained.

The results of the current study showed that both Quat™ 128 and Quat™ 256 are effective in killing adult and veliger dreissenids in all testing conditions. Adult dreissenids, although rare, can be found in various components of wildland firefighting equipment such as engines, water tenders, and aircraft. In laboratory conditions, adult *D. rostriformis bugensis* reached 100% mortality by 48 h when exposed to 1, 3, and 5% Quat™ 128 and 36 h when exposed to 1, 3, and 5% Quat™ 256, whereas adult *D. polymorpha* reached 100% mortality within 6 h when exposed to all treatment groups of Quat™ 128 and Quat™ 256. The results suggest that adult *D. polymorpha* are more susceptible to Quat™ 128 and Quat™ 256 when compared to adult *D. rostriformis bugensis*. This could be attributed to the fact that *D. rostriformis bugensis* is a more competent species. Compared to *D. polymorpha*, *D. rostriformis bugensis* are thought to be more competitive and are displacing *D. polymorpha* in the Great Lakes as *D. rostriformis bugensis* have higher filtration rates and assimilation efficiency and a lower

respiration rate, which saves energy for growth and reproduction, higher growth rates, and a smaller portion of body tissue for reproduction (Diggins, 2001; Baldwin et al. 2002). In laboratory settings, *D. rostriformis bugensis* can survive, grow, and feed as well or better than *D. polymorpha* (Baldwin et al., 2002).

When using Quats to decontaminate wildland firefighting equipment, it is crucial that it works quickly. Fire suppression equipment can be re-enlisted immediately after a fire incident, and if the equipment needs to be decontaminated for up to two days, quats may not be a viable option. If the equipment was decontaminated long enough to kill all mussels, the shells and debris would still need to be removed to ensure it will work properly. If this equipment has grown mussels inside it should be pulled out of the field and taken apart to be manually decontaminated.

This study mainly focused on the effects of Quat™ 128 and Quat™ 256 for decontaminating wildland firefighting equipment contaminated with dreissenid veligers as opposed to adults because veligers are more likely to contaminate this equipment. There was not much of a difference between veliger species when exposed to Quat™ 128 and Quat™ 256. Quat™ 128 and Quat™ 256 induced rapid mortality in dreissenid veligers within minutes. After 1 min of exposure to 0.5% Quat™ 128 and 0.1 and 0.25% Quat™ 256, *D. rostriformis bugensis* and *D. polymorpha* reached 100% mortality at ambient room temperature (21°C). Other studies have been conducted using Sparquat 256®, which is similar to Quat™ 256, but with less of the active ingredients. The active ingredient in Sparquat 256® is a blend of 10% DDAC and ADBAC, whereas the active ingredient in Quat™ 256 is a blend of 16.9% DDAC and ADBAC (Buckeye International, 2006). One study reported 100% mortality in *D. rostriformis bugensis*

using 3% Sparquat 256® after a 10 min exposure time (Britton & Dingman, 2011). This was a relatively small study design where only 89 veligers were used. The intention of the researchers was to conduct a quick, preliminary test to evaluate using Sparquat 256® as a means for decontaminating wildland firefighting equipment.

Other AIS have been tested against Sparquat 256® with promising results. For instance, 3% Sparquat 256® is effective in treating equipment exposed to whirling disease and New Zealand mudsnails in less than 15 min (Hedrick et al, 2008; Schliser, Vieira, & Walker, 2008). However, since these studies have been published, Sparquat 256® is no longer available on the market and Green Solutions High Dilutions 256®, with the same concentration of the active ingredient is used as a replacement (U.S. FS, 2012).

Quat™ 128 has not been previously tested on dreissenid veligers. However, 4.4% and 4.6% Quat™ 128 has been successful in less than 15 min when treating equipment exposed to whirling disease and New Zealand mudsnails, respectively (U.S. FS, 2012). The active ingredient in Quat™ 128 is a blend of 8.45% DDAC and ADBAC, which is half the concentration of the same active ingredient as Quat™ 256 (16.9%). This shows that Quat™ 256 is stronger at decontaminating equipment exposed to AIS, especially *D. rostriformis bugensis* and *D. polymorpha* and was verified in the current study. In all study conditions, veligers died at a faster rate when exposed to Quat™ 256 as opposed to the treatment groups exposed to Quat™ 128 concentrations.

Previous studies using quats to kill AIS have been conducted in the laboratory, with indoor, ambient air temperatures and varied water temperatures. The current study took into account differing water and ambient air temperatures to ensure more parameters

are covered when making recommendations for decontaminating wildland firefighting equipment. Because places to decontaminate this equipment can vary from indoors to outdoors, from hot and dry to cold and humid, water and ambient air temperatures, such as 2, 16, and 30°C, and 2, 16, 30, and 43°C, respectively, were used to evaluate the effectiveness of killing dreissenid veligers.

Depending on where the decontamination station is stored, the chemical solution temperature can vary. Therefore, the tested water and air temperatures include a wide range of temperatures represented in fire incidents nationwide. In the present study, rapid veliger mortality was observed with the combined variables of water temperature and chemical exposure. The results are not surprising in that *D. rostriformis bugensis* veligers seem to be more susceptible to the warmer water temperatures and chemical solution combinations when compared to *D. polymorpha*. The upper thermal limit for *D. rostriformis bugensis* is lower than that of *D. polymorpha* (Spidle et al., 1995; McMahon, 1996; Mills et al., 1996). While these studies were conducted on adult dreissenids, the results of this study verify that the same is true for veligers. *D. rostriformis bugensis* veligers reached 100% mortality in all Quat™ 128 and Quat™ 256 treatment groups when exposed to water temperatures at 30°C for 1 min. Conversely, *D. polymorpha* reached 100% mortality when exposed to 0.25 and 0.5% Quat™ 256 for 5 min.; hence, *D. polymorpha* are less susceptible to the warmer water temperature and chemical solutions compared to *D. rostriformis bugensis*. Solutions of Quat™ 128 combined with 30°C water temperature was not able to induce 100% mortality in *D. polymorpha*.

Both species did not reach 100% mortality when exposed to 2°C of 0.25% Quat™ 128 and 0.1% Quat™ 256 after 1 min of exposure. When temperatures are lower, the

mussel's metabolism decreases, especially when they are facing a threat. The mussels save energy as a means of self-protection. It seems as though the combination of water temperature and chemical solution work synergistically to kill dreissenid veligers. This is seen more in the upper (30°C) and lower (2°C) water temperatures as opposed to the 16°C treatment groups. This is a water temperature where both *D. rostriformis bugensis* and *D. polymorpha* do very well in regards to survival and reproduction (McMahon, 1996). It is likely that the mortality seen in this group is because of the toxicity alone that Quats™ 128 and 256 induced.

Finally, the decontamination solutions most likely would not be made up for each use; therefore, the chemical solutions were tested for 1, 5, and 10 days to see if the potency to kill *D. rostriformis bugensis* veligers would be reduced as time increased. Fortunately for the U.S. FS, the length of storage time of the Quat™ 128 and Quat™ 256 solutions does not seem to be a factor and does not have an effect on the ability of the chemical to kill dreissenid veligers. The current study found that 0.25% Quat™ 128 will induce 100% mortality in *D. rostriformis bugensis* veligers when exposed to 40 min duration. This is after the chemical solution was stored at 2, 15, 30, and 43°C for 1, 5, and 10 days. Likewise, veligers exposed to 0.1% Quat™ 256 induced 100% mortality after 40 min of exposure in the same conditions. These results suggest that Quat™ 128 and Quat™ 256 are not affected by ambient temperatures, ranging from 2°C to 43°C, and solutions can be used up to ten days to decontaminate wildland firefighting equipment. Currently, there are not any other studies in the literature testing the effectiveness of Quat™ 128 and Quat™ 256 when exposed to varying ambient temperatures.

Discussion of Research Questions

This dissertation was designed to answer an array of questions pertaining to preventing the further spread of *D. rostriformis bugensis* and *D. polymorpha* though decontamination methods. First, the objective of this study was to determine if there was any difference in mussel species when removing them from watercraft. The results from the study indicated that there is no difference in *D. rostriformis bugensis* and *D. polymorpha* in regards to using pressurized water spray for removal from watercraft. The next objective of the study was to determine the most effective psi of water for removing dreissenids from watercraft and the time it would take to remove those mussels. Also, the study attempted to determine the ease of removing dreissenids from a watercraft that has been stored out of the water for more than one week compared to being fresh out of the water. The results show that 3000 psi of water is the most effective pressure to use especially if the watercraft is fresh out of the water (week 0); therefore, the null hypotheses in both cases can be rejected. However, 1500 psi of water will suffice to remove high density druses of dreissenids in 8.0 ± 2.3 s for *D. rostriformis bugensis* and 15.6 ± 11 s for *D. polymorpha* if the watercraft has been out of the water more than one week in the summer and 18.83 ± 9.32 s for *D. rostriformis bugensis* if the watercraft has been out of the water for more than four weeks, respectively (Tables 11 & 12).

Finally, this study attempted to assess the shortest amount of time needed to attain 100% mortality of *D. polymorpha* following exposure to hot-water spray, and also, if that time and temperature would be the same to kill *D. rostriformis bugensis*. The results showed that *D. polymorpha* exposed to 54°C for 10 s and 70°C for 5 s was sufficient to reach 100% mortality. The null hypothesis that *D. rostriformis bugensis* will not be more

susceptible to hot-water spray than *D. polymorpha* can be rejected because Comeau et al. (2011) found that *D. rostriformis bugensis* exposed to hot water spray temperatures of 60°C for 5 s and 54°C for 10 s reached 100% mortality. Their results show that *D. rostriformis bugensis* is more susceptible to hot water spray because it takes less time to reach 100% mortality compared to *D. polymorpha*. If a mussel fouled watercraft arrives at an inspection station, and the species is unknown, using the results from the combined research, it is best to decontaminate the vessel with 54°C for 10 s.

Lastly, this dissertation attempted to answer several questions in regards to evaluating the effectiveness of Quat™ 128 and Quat™ 256 on killing adult and veliger dreissenids for decontaminating wildland firefighting equipment. The results found in this study show that both Quat™ 128 and Quat™ 256 are effective in killing adult and veliger *D. rostriformis bugensis* and *D. polymorpha* in low concentrations, with veligers being far more vulnerable to the chemical solutions, and died much quicker compared to the adult dreissenids. The null hypothesis can be rejected because *D. rostriformis bugensis* and *D. polymorpha* veligers are more susceptible to Quat™ 128 and Quat™ 256 compounds compared to adults. Adult *D. rostriformis bugensis* experienced mortality after 36 h of exposure to all treatment groups of Quat™ 256 and after 48 h of exposure to all treatment groups of Quat™ 128. *D. polymorpha* experienced 100% mortality after exposure to all treatment groups of Quat™ 128 and Quat™ 256. Dreissenid veligers showed rapid mortality in just minutes when exposed to Quat™ 128 and Quat™ 256. After 1 min of exposure to 0.5% of Quat™ 128, in ambient room temperature, all dreissenid veligers died. After 1 min of exposure to 0.1% of Quat™ 256 and 0.25% of

Quat™ 256, *D. rostriformis bugensis* and *D. polymorpha* veligers reached 100% mortality, respectively.

The study also attempted to find out if *D. rostriformis bugensis* is more or less susceptible than *D. polymorpha* to Quat™ 128 and Quat™ 256 treatment. Although there are a lot of similarities between *D. rostriformis bugensis* and *D. polymorpha* in their natural history (Mills et al., 1996), the ecological and physical characteristics between them are different. Compared to *D. polymorpha*, *D. rostriformis bugensis* are thought to be more competitive and are displacing *D. polymorpha* in the Great Lakes as quagga mussels have higher filtration rates and assimilation efficiency and a lower respiration rate, which saves energy for growth and reproduction, higher growth rates, and a smaller portion of body tissue for reproduction (Diggins 2001; Baldwin et al. 2002). The results of this study showed that adult *D. polymorpha* is more susceptible to chemical solutions of Quat™ 128 and Quat™ 256. However, veliger *D. rostriformis bugensis* reached mortality at a faster rate when exposed to chemical solutions of Quat™ 128 and Quat™ 256 when compared to *D. polymorpha*.

Finally, the study attempted to examine the effects of water temperature and air temperature on the effectiveness of Quat™ 128 and Quat™ 256 on killing dreissenid veligers. The results indicate that the null hypothesis cannot be rejected in that water and air temperature do not have an effect on the effectiveness of Quat™ 128 and Quat™ 256. It seems as though warmer water and air work synergistically with the chemical compounds to induce mortality. Also, the length of storage time of the Quat™ 128 and Quat™ 256 solutions does not seem to be a factor and does not have an effect on the chemical's ability to kill dreissenid veligers. These results are positive in the fact that the

concentration of the chemical can be trusted and used to decontaminate wildland firefighting equipment in most situations.

Study Limitations

There were several study limitations with regards to all the evaluations presented within this dissertation. First, the project that analyzed pressurized water spray to remove dreissenids on watercraft was never fully completed, as data for removing *D. polymorpha* in the winter season were never collected. The first attempt at collecting these data was in December 2012 at Milford Lake, Kansas. Because of equipment malfunctions, the project was not carried out to completion and it was put on hold until the next winter season. The following year was a long, cold winter. Milford Lake remained frozen until the end of April. By that time, spring conditions were too warm to conduct the experiment and the results would not be conducive to winter.

The sample areas selected to remove dreissenids from watercraft were of convenience. Most of the samples came from the hull of the watercraft where it was easy to section them off and the researcher was able to remain in a comfortable position for extended periods of time. Watercraft comes in all different sizes and there are so many nooks and corners on vessels that are difficult to reach. For instance, the times reported in this dissertation may be extended when using high pressure spray to remove mussels on the lower unit or in areas that are difficult to reach.

Second, the results for evaluating the susceptibility of *D. polymorpha* to hot water spray boat validation was completed using juveniles (treatment mussels: 8.16 mm, range = 4.84-14.26 mm and control mussels: 8.15 mm, range = 6.17-14.15 mm). Many of the

vessels at Wilson Lake are pulled out for the winter season; therefore, by August, the sizes of the mussels attached to watercraft have not reached full maturity.

Lastly, the results observed in the analyses of the lethal effects of Quat™ 128 and Quat™ 256 on killing adult and veliger dreissenid studies should not be assumed to apply in all situations because of the *in vitro* nature of the study. Specific water and ambient air temperatures were selected to be tested with the quats to evaluate their effectiveness on killing dreissenids. However, tests were not conducted to evaluate ranges of water and air temperatures. The results only apply to specific testing parameters. Also muddied or diluted chemical solutions were not evaluated. The results indicated that Quat™ 128 and Quat™ 256 chemical solutions can be used up to ten days without losing potency; however, if for instance, the U.S. FS is using the decontamination solutions every day, it can become diluted. When chemical solutions were tested at differing ambient temperatures, the solutions were prepared in Nalgene bottles with air tight lids so that evaporation would not be an issue.

Study Contributions

There are numerous ways to prevent and control new *D. rostriformis bugensis* and *D. polymorpha* infestations. Humans and human activities are the primary means in which dreissenids spread. Decontaminating watercraft with attached mussels, using high pressure and hot water spray, is one of the best ways to prevent to dreissenids from spreading. Vessels that have been in the water for more than a few days may have veligers or juvenile mussels attached to their hulls, anchors, lines, engines, and other equipment. Boat inspection and decontamination are important elements of a

monitoring program. The data from this dissertation can be used in the development of an inclusive, standard protocol for killing and removing 100% of both species of dreissenid mussels for watercraft for all states, agencies, tribes, and private entities in the U.S. Currently, the combination of high pressure and hot water spray is the most effective and sustainable method to kill and remove mussels from watercraft. Water is eco-friendly, relatively inexpensive, and is an easily accessible resource with low application time. When procedures and standards are followed correctly, watercraft decontamination by means of high pressure and hot water sprays can be effective at preventing the spread of *D. rostriformis bugensis* and *D. polymorpha*.

Wildland firefighting equipment is categorized as a secondary means of transporting dreissenids and other AIS. Contaminated equipment can act as vectors moving contaminated water around the landscape, which creates pathways for invasion. If equipment is not completely drained or treated, that equipment can transport *D. rostriformis bugensis* and *D. polymorpha* to mussel-free watersheds and water-bodies, including remote and isolated headwaters. The new introduction could lead to a new infestation. The results observed in the analyses of the lethal effects of Quat™ 128 and Quat™ 256 on killing adult and veliger dreissenid studies can provide baseline data on the development of a standard and effective decontamination protocol on firefighting equipment exposed to *D. rostriformis bugensis* or *D. polymorpha* throughout the country and in particular in the western U.S.

Recommendations for Further Study

The work done in this dissertation can be used to create standards in dreissenid decontamination protocols. However, it did not cover everything and gaps still exist. More research needs to be done to examine if 6, 7, 8, or 9 s is sufficient to kill *D. polymorpha* at 60°C. The current study looked at 5 and 10 s. There is a real possibility that a time frame in the middle would work as well. This may save on time to kill mussels attached to watercraft.

There is not a lot of research in literature that has examined the use of Quat™ 128 and Quat™ 256 in killing dreissenid veligers. More work needs to be conducted to see if Quat™ 128 or Quat™ 256 can be used in closed systems for deterring veliger settlement on infrastructure. The current study was completed in the laboratory and none of the Quat™ experiments have been validated in the field situation. More extensive research needs to be done to evaluate the long term effects of wildland firefighting equipment exposed to Quat™ 128 and Quat™ 256 for extended periods of time.

Overall, there are gaps in inspection and decontamination programs. Some agencies and water bodies do not allow a vessel to launch if there is standing water on board. To allow more boaters to boat and be less of an inconvenience, more research needs to be done to measure how long and how many veligers can live in the standing water of a vessel's engine. This is an area where all the water can never be completely drained, so having the knowledge and understanding the risk of water in the engine may help inspection and decontamination programs to allow more boaters to boat while not increasing the biological risk of introducing an invasive species.

Conclusions

Through the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990, *D. polymorpha* was designated as an injurious species under the Lacey Act[1] to restrict intentional transport of this species into and throughout the U.S. Unfortunately, when this law was written, *D. rostriformis bugensis* was not an issue and it is not listed an injurious species. This means that law enforcement cannot issue citations for people that transport this species across state lines. This is an issue primarily in the West as *D. rostriformis bugensis* is the dominant species.

Climate change is a global stressor which can be a contributing factor in exacerbating the expansion of AIS, as the severity of extreme weather events can facilitate movement of such species. A shift is climatic variables favors species with larger bioclimatic ranges, and these changes are increasing the vulnerability of some freshwater habitats (Hickey, 2014). Over the past 100 years, at least 162 species have been introduced into the Laurentian Great Lakes (Ricciardi, 2001). The majority of these species were unintentionally introduced through ballast water discharges, canal construction, and accidental releases from aquaculture escapes and aquaria. With the NANPCA of 1990, new species introductions were being controlled. For instance, ships coming into the region are required to exchange their ballast water at least 200 miles away (Griffiths, Schloesser, & Kovalak, 2013). Unfortunately, the Canadian government did not issue similar mandatory, ballast water regulations. Grigorovich et al. (2003) identified over 40 species that could be introduced into the Great Lakes via ballast water discharge. One of the threat risk species is a native of Southeast Asia, *Limnoperna*

[1] The Lacey Act of 1900 (16 U.S.C. 3371-3378) was one of the earliest laws with provisions against transportation and unpermitted possession of invasive (injurious) animal species, including fish.

fortune, also known as the golden mussel. The golden mussel has caused similar problems of *D. rostriformis bugensis* and *D. polymorpha* such as ecological and biofouling impacts in its region (Boltovskoy et al. 2009). AIS are difficult to control and until every federal, state, local, and private agency all come together on this issue, containment programs will have gaps and *D. rostriformis bugensis* and *D. polymorpha* and other AIS will continue to spread. The findings of this dissertation can be used to assist in closing inspection and decontamination program gaps by helping to create standardized protocols in dreissenid decontamination (APPENDIX E). These standardized protocols will be given the WRP for evaluation. The goal is to disseminate all the results and practical applications to all AIS coordinators and lake managers in the Western U.S.

APPENDIX A—KANSAS DEPARTMENT OF WILDLIFE, PARKS AND TOURISM COLLECTION PERMIT

Kansas
Department of Wildlife, Parks and Tourism

Kansas ANS Possession Permit

K.A.R. 115-18-10
Authorization Statues:
K.S.A. 32-807
K.S.A. 32-956

KAquatic **N**uisance **S**pecies **A** **S**

Permit Number: ZM-5
Effective: Date of signature below
Expires: October 1, 2011
Non-renewable

PERMITTEE:
Dr. David Wong
Ashlie Watters
4505 Maryland Pkwy
Las Vegas, NV 89154
702-277-5961

PERMIT TYPE:
Possession of zebra mussels

CONDITIONS AND AUTHORIZATIONS:

A. Authority is hereby granted for permittee to possess live zebra mussels *Dreissena polymorpha* for research purposes.

B. Procedures outlined in application must be followed to ensure zebra mussels do not become established in the wild.

C. Live zebra mussels must be held in a container clearly labeled indicating that zebra mussels and contaminated water are present and this permit must accompany the specimens.

C. All live zebra mussels will be eliminated upon expiration of permit. Remaining live zebra mussels and infested water should be remitted to Kansas Department of Wildlife, Parks and Tourism, 512 SE 25th Ave, Pratt, KS 67124 for disposal.

D. Permit may be revoked at any time.

Keith Sefton — 8-1-11
ISSUED BY: DATE:

APPENDIX B— NATIONAL PARK SERVICE COLLECTION PERMIT

SCIENTIFIC RESEARCH AND COLLECTING PERMIT Grants permission in accordance with the attached general and special conditions **United States Department of the Interior** **National Park Service** **Lake Mead NRA**	**Study#: LAME-00181** **Permit#: LAME-2010-SCI-0018** **Start Date: Oct 01, 2010** **Expiration Date: Dec 31, 2010** **Coop Agreement#: n/a** **Optional Park Code: n/a**

Name of principal investigator:

Name: David WH Wong **Phone:** 702-8952446 **Email:** David.Wong@unlv.edu

Name of institution represented:

University of Nevada - Las Vegas

Co-Investigators:

Name: Ashlie Watters **Phone:** 702-277-5361 **Email:** wattersa@unlv.nevada.edu

Project title:

Protocol for Testing the Effectiveness of an Algaecide, EarthTec, on Killing and Preventing the Colonization of Quagga Mussels

Purpose of study:

Purpose of the proposed project is to test an US EPA registered algaecide on killing quagga mussels, invasive species in the Southwest that have been threatening the local ecosystems and enviroments.

Subject/Discipline:

Exotic / Invasive Animals

Locations authorized:

Quagga mussels will be collected from Las Vegas Boat Harbor

Transportation method to research site(s):

Samples will be collected with hand

Collection of the following specimens or materials, quantities, and any limitations on collecting:

n/a

Name of repository for specimens or sample materials if applicable:

n/a

Specific conditions or restrictions (also see attached conditions):

If samples are collected from cultural resource sites, care must be taken to insure cultural resources are not damaged during the removal process.

Recommended by park staff(name and title):

Reviewed by Collections Manager:

Yes ___ No ___

Approved by park official:

Date Approved:

10/20/2010

Title:

Chief, Resources Management

I Agree To All Conditions And Restrictions Of this Permit As Specified
(Not valid unless signed and dated by the principal investigator)

(Principal investigator's signature)

10/25/2010
(Date)

THIS PERMIT AND ATTACHED CONDITIONS AND RESTRICTIONS MUST BE CARRIED AT ALL TIMES WHILE CONDUCTING RESEARCH ACTIVITIES IN THE DESIGNATED PARK(S)

Collecting Water Samples For *Dreissena* spp. Veliger PCR Analysis

Bureau of Reclamation
Technical Service Center
Denver, Colorado

Equipment Needed:

- 63-µm Plankton Tow Net (Mesh size is critical). (We use custom Wildco plankton net with a 500 mm-diameter opening, flow meter (optional), and a 2-m length.)
- Spray Bottle – 1-L
- Ethanol (lab grade, 200 proof; or from a local liquor store, e.g., Everclear 190 proof =95% or Rum 151 proof = 75.5%)
- Sample Bottles (1000-mL Nalgene leak-proof poly (HDPE))
- Disposable Diapers
- Plastic electrical tape
- Ziploc Bags – 1-gal.
- Plastic Garbage Bags (large enough to hold 4 sample bottles)
- Waterproof Markers and Labels
- Data Sheet and Waterproof paper
- Ice chest with cubed/crushed ice or frozen "blue ice"
- Decontamination container for sampling net (e.g., ½ plastic barrel with inside diameter greater than plankton net hoop to permit complete submersion)
- White vinegar (from grocer) or 5% acetic acid solution - 12-16 L (i.e., enough to cover plankton net in decontamination container)

Sample Collection Procedures:

1. Introduction - These procedures are designed to collect the veligers or the free-swimming larval form of zebra and quagga mussels (*Dreissena* spp.) as plankton samples for laboratory detection using polymerase chain reaction (PCR). Step-by-step collection procedures are included below. The volumes of water sampled through the plankton net are needed both for sample size standardization and for calculating the number of veliger density by microscopic methods to confirm the PCR results. Collect a minimum of two replicate plankton samples at each location.

Note: If the plankton net has been contaminated with zebra or quagga mussel veligers from previous collection events, it should be decontaminated with acetic acid (vinegar) and rinse prior to sample collection. Go to Steps 6-8 for this procedure. Save the final water rinsate sample for laboratory analyses to confirm decontamination. Record and label information about the rinsate (Step 5).

2. There are two methods of acquiring the water sample:
    a. Plankton net tow – Lower the net to the desired, measured depth and slowly tow it for a known recorded distance. The volume of water that is sampled can be determined based on the diameter of the net opening and the distance towed. A minimum sample volume of 1,000 L is recommended. Record: Depth and distance of the tow.
    (Caution: To assure accuracy of the sample volume, do not let the retrieval speed exceed the filtration rate of the net.) Remember that veligers from spawning zebra and quagga mussels are more commonly found in deeper water so sample accordingly. Go to Step 3.
    b. Pumped source – This may be taken either by a portable pump from a boat or from the raw, untreated water plumbing system of a dam or water treatment plant. Open the flow valve and completely purge the supply line of any stagnant water. If a flow meter is not available on the pipe, use a five gallon bucket and a second timer to determine the flow rate (gallons per minute) through the pipe. Calculate the mean of at least 3 replicate runs for determining the flow rate. Place the plankton tow net under the hose and collect all of the water flowing out of the valve and keep an accurate measure of the volume of water flowing into the net by recording the elapsed time. A minimum of 1,000 L must pass through the net. Record the total volume of filtered water collected per sample and the water depth of the intake of the water source. Go to Step 3.

3. Using water, wash down the net from the outside to concentrate veligers into the collection cup. Carefully unscrew the collection cup and pour the sample into a 1000-mL Nalgene leak-proof poly bottle. Thoroughly rinse the collection cup with spray bottle with minimal volume of water and transfer the rinses into the same sample bottle. Take care to keep the wash and/or rinse water away from the opening of the plankton net and wash only along the outside of the plankton net and cup, so that the filtered volume remains unchanged. MARK THE WATER LEVEL ON THE SAMPLE BOTTLE WITH PERMANENT INK (Draw a line on the bottle and label “Level 1).

4. Add an appropriate volume of ethanol to get 25% final concentration in the sample bottle (visually estimate, does not have to be exact). For example, if using lab grade ethanol or 190 proof Everclear, use 3 parts lake water and 1 part etEverclear. Replace bottle cap snugly. (Note: The volume of ethanol will be needed in the calculation of number of veligers per unit volume; therefore be sure that the sample bottle is marked with a second line to indicate total volume (sample + ethanol) so that the lab can also determine the volume of ethanol that was added.) Draw a line on the bottle and label “Level after ETOH”. Tape the secured bottle cap with black electrical tape to cover the seam between the cap and bottle to prevent leakage. Wrap the bottle in a disposable diaper and place in a Ziploc bag (push all air out of bag before closing). Put both the replicates from

same location into one single plastic garbage bag. Put on ice in cooler for transport.

5. Labeling sample bottles. Use waterproof Sharpie pens for bottle labels and mechanical pencils for data sheets. Be careful to avoid spillage of ethanol – Sharpie ink will run if contacted with ethanol. For backup, record sample bottle information with a mechanical pencil on a piece of waterproof paper and insert paper into the Ziploc bag along with the sample bottle. Record the following information on both sample bottle and data sheet:

    - Sample Date
    - Sample Location (GPS if available, otherwise describe location – i.e. near north shore boat dock, etc.)
    - Sample depth or intake depth in water column
    - Volume of water filtered through the plankton net Mark sample poly bottle with two lines of permanent ink, one for level of sample and one for total level of sample + ethanol
    - Preservative used (e.g., 25% ethanol)
    - Name of person collecting sample with contact information (phone number)

6. Veligers easily stick to the walls of the plankton net. Decontamination (and disinfection) is critical to avoid cross contamination from one sample location or event to another and possibly the spread of mussels to new waters. It is recommended that each sampling location (reservoir) has a dedicated collection net. Each time the net is used at a new sample site, the procedure will require a soak treatment in a 5% v/v acetic acid bath. A 5% acetic acid solution may be purchased as white vinegar, or a 5% solution may be prepared with concentrated (glacial) acetic acid and water. These steps will both denature the DNA for the PCR process and dissolve the veliger shells otherwise visible in microscopic observations.

7. The recommended treatment for the plankton net following sample collection is to first rinse the net with clean water to wash as many veligers from the net as possible, and then totally immerse the net in the 5% acetic acid bath. The ideal soak time is overnight; however, if it is necessary to use the net at the next sampling location during the same day, a one hour soak followed up with a rinse prior to the next sampling should be the minimum. The same acetic acid bath may be used repeatedly for all sample sites. Following the acetic acid soak, rinse the net with a large volume of clean water (e.g., 100 L) allowing the rinse water to drain and collect into the collection cup.

8. Pour the collected rinsate into a sample bottle, preserve with ethanol, and labeled as directed in Steps 4 and 5. The final rinsate from each sample location may be combined at the end of the day and sent as one sample. Ship on ice with the other samples at the address given.

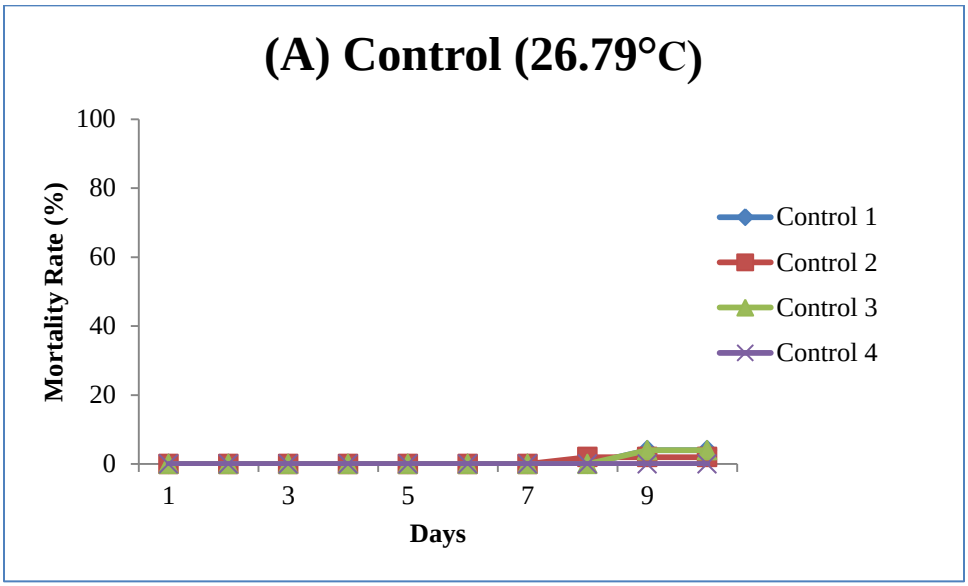
9. Keep samples cool at all times. Samples may be stored under refrigeration for a few days if a delay is necessary to avoid shipping over a weekend.

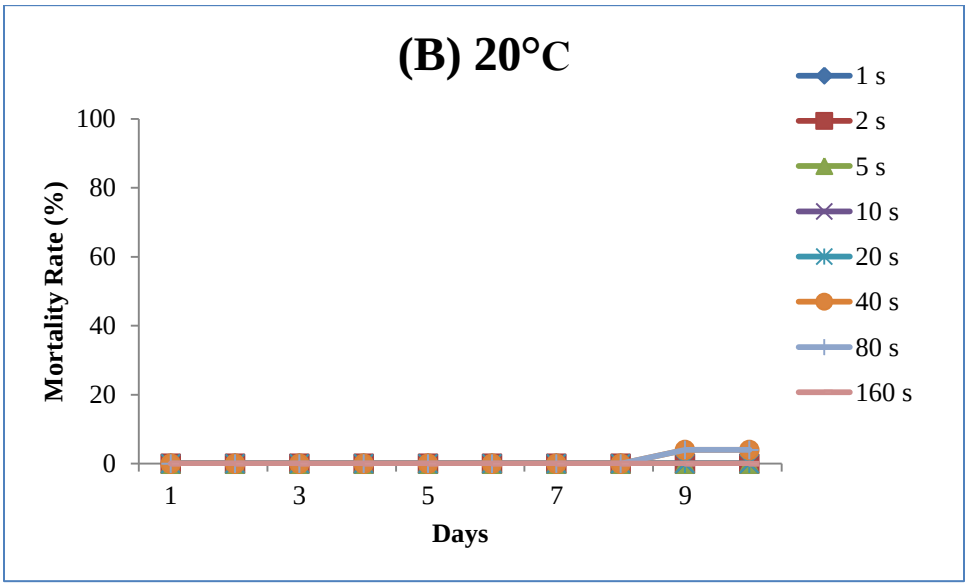
10. **Ship samples using FedEx Overnight Express (AVOID WEEKEND DELIVERIES!) to:**

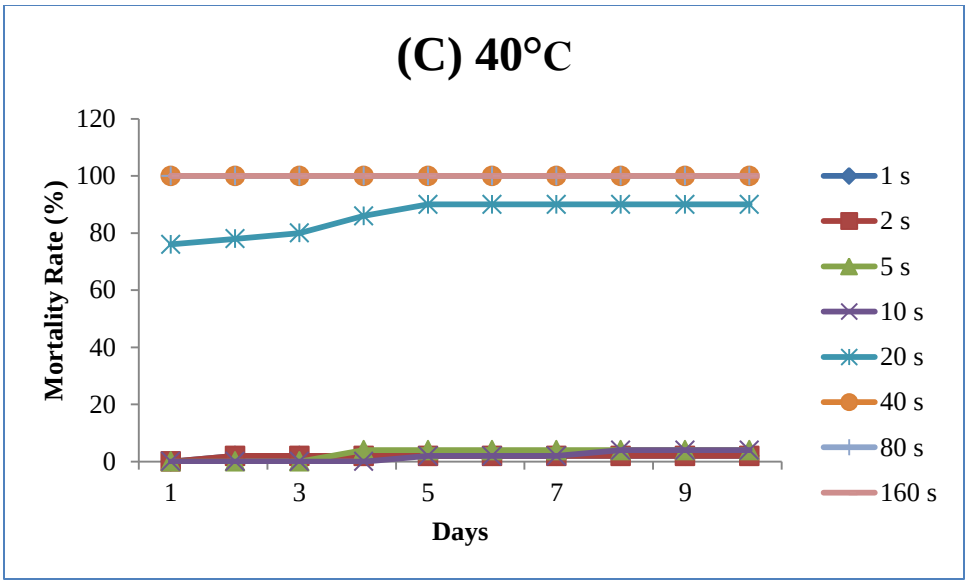
    Kevin Kelly/Denise Hosler (86-68220)
    U.S. Bureau of Reclamation
    Denver Federal Center
    Corner of 6th Ave. & Kipling
    Bldg 67, Room 152
    Denver, CO 80225-0007
    Contact information:
    Kevin Kelly: kkelly@do.usbr.gov
    Denise Hosler: Phone: (303) 445-2195; dhosler@do.usbr.gov
    Fred Nibling: Phone: (303) 445-2202; fnibling@do.usbr.gov

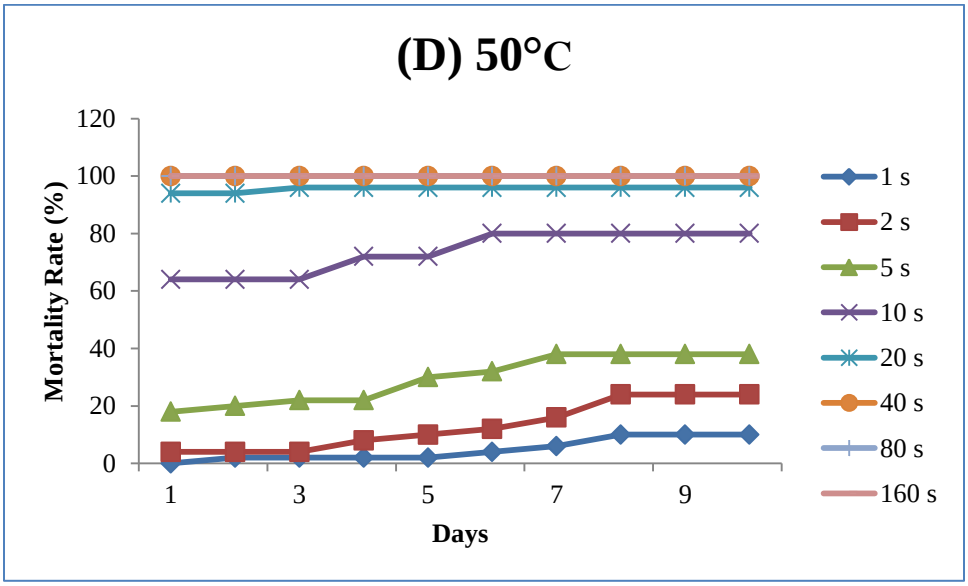
APPENDIX D—HOT-WATER SPRAY TREATMENT FIGURES

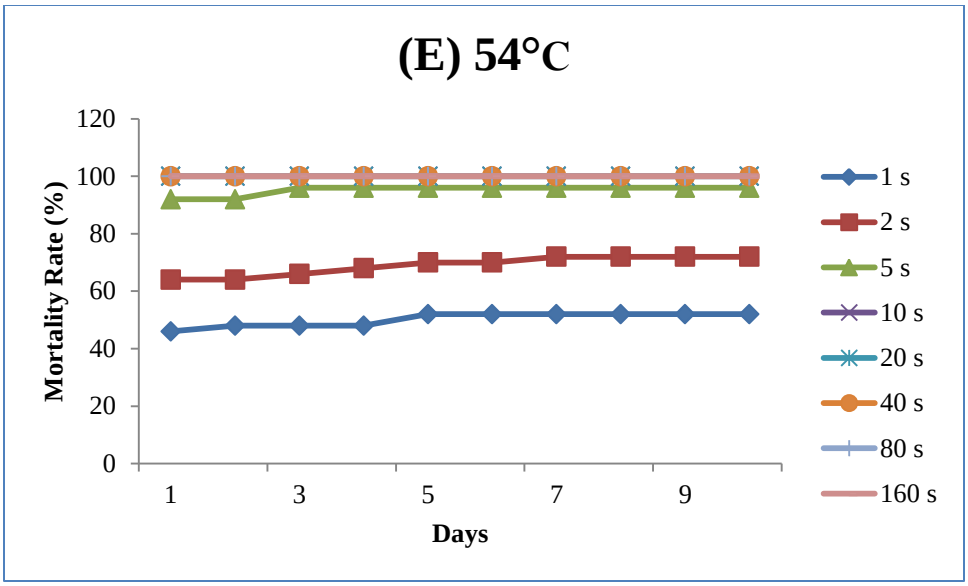
Figures of mortality rates (%) of *D. polymorpha* in Wilson Lake, KS after hot-water spray treatment. (a) Control (26.79°C); (b) 20°C; (c) 40°C; (d) 50°C; (e) 54°C; (f) 60°C; (g) 70°C; (h) 80°C

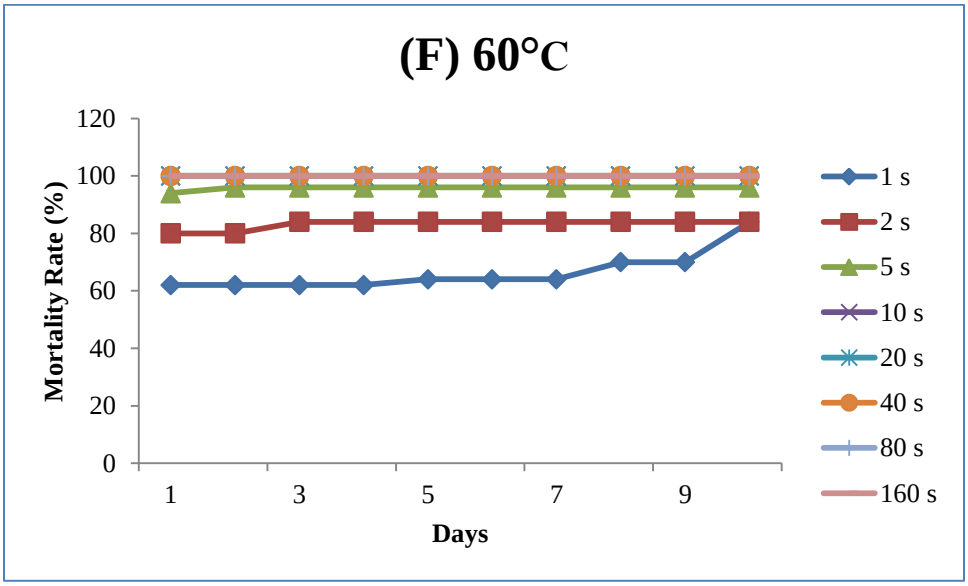


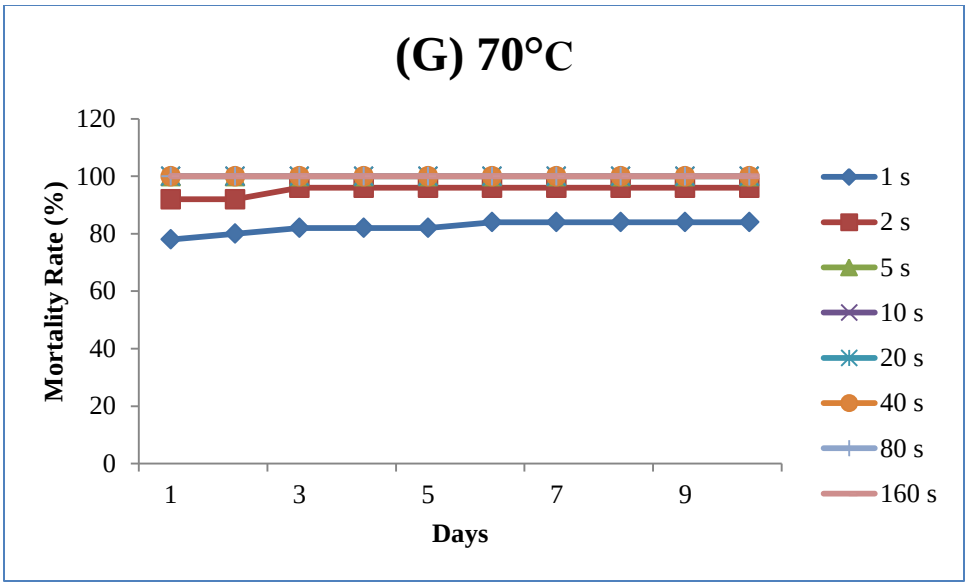


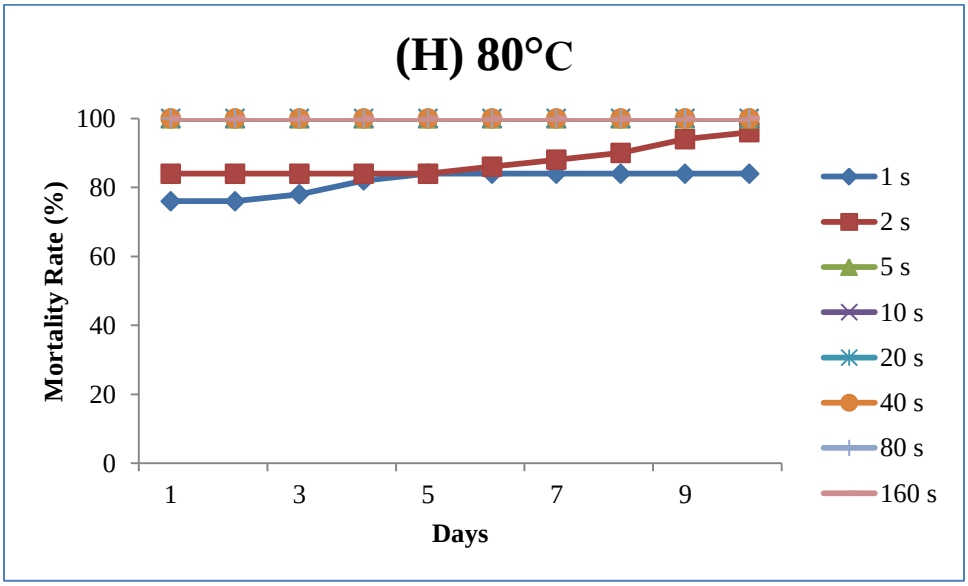

(C) 40°C
Mortality Rate (%)
120
100
80
60
40
20
0
1
3
5
7
9
Days
1 s
2 s
5 s
10 s
20 s
40 s
80 s
160 s


(D) 50°C
Mortality Rate (%)
120
100
80
60
40
20
0
1
3
5
7
9
Days
1 s
2 s
5 s
10 s
20 s
40 s
80 s
160 s


(E) 54°C
Mortality Rate (%)
120
100
80
60
40
20
0
1
3
5
7
9
Days
1 s
2 s
5 s
10 s
20 s
40 s
80 s
160 s


(F) 60°C
Mortality Rate (%)
120
100
80
60
40
20
0
1
3
5
7
9
Days
1 s
2 s
5 s
10 s
20 s
40 s
80 s
160 s


(G) 70°C
Mortality Rate (%)
120
100
80
60
40
20
0
1
3
5
7
9
Days
1 s
2 s
5 s
10 s
20 s
40 s
80 s
160 s


(H) 80°C
Mortality Rate (%)
100
80
60
40
20
0
1
3
5
7
9
Days
1 s
2 s
5 s
10 s
20 s
40 s
80 s
160 s

APPENDIX E—STANDARDIZED PROTOCOL

This research evaluated three techniques for preventing the further spread of dreissenids: 1) high pressure water sprays to remove dreissenids from watercraft, 2) hot-water spray to kill *D. polymorpha*, and 3) use of quaternary ammonium compounds, Quat™ 128 and Quat™ 256 for decontaminating wildland firefighting equipment. The aim of decontaminating watercraft and equipment, including wildland firefighting equipment, is to reduce the biological risk of spreading AIS, in particular dreissenids.

The results of this dissertation show that it is best to use 3000 psi of water on the hull, centerboard box and keel (sailboats), lower unit, cavitation plate, and prop. These external areas can handle 3000 psi of water on most watercraft without causing damage and usually have the most amount of mussel fouling. For internal and other sensitive areas of watercraft, manual removal, using brushes and scrapers, of mussels may be necessary. For personal safety, only trained personnel should use high pressure water spray to remove dreissenids from watercraft. Using 3000 psi of water, as opposed to 1500 psi of water, to remove dreissenids from watercraft is accomplished at a faster rate when the vessel has been out of the water for at least one week in the summer and two weeks in the winter compared to being fresh out of the water (week 0).

The results of this study found that *D. polymorpha* are susceptible to hot-water spray. To reach 100% mortality, 54°C for 10 s should be used for *D. polymorpha* and another study by Comeau et al. (2011) found that *D. rostriformis bugensis* reached 100% mortality within 10 s when exposed to 54°C as well, or 5 s when exposed to 60°C water temperatures. When the species is unknown, it is recommended to use 54°C for 10 s to ensure complete mussel mortality. Combining the methods of pressurized (3000 psi of

water) and hot-water spray (54°C for 10 s) to the surface of the fouled watercraft is the best way to decontaminate a vessel to prevent the further spread of dreissenids. For the inaccessible areas, such as the gimbal area, inside the engine, generator and AC cooling systems, treatments of 54°C for 10 s will not suffice. According to Comeau et al. (2011), the amount of time needed to achieve the target lethal temperature is 43 s for the summer time, and 2 minutes and 7 s for the winter time. The hot water needs to warm up these internal compartments. In addition, most watercraft have special areas that have water transfer pumps that can handle water temperature ≤ 49°C for decontamination, such as ballast tanks/bladders, wash-down systems, bait and live wells, and internal water systems. This temperature is adequate to kill dreissenid veligers. For these sensitive areas, it is recommended that the temperature of the hot-water flush be monitored until a temperature of 49°C is reached. After this target temperature is reached, it is necessary to maintain a constant flush of that temperature for at least 10 s to ensure 100% mussel mortality (Comeau et al., 2011).

Again, it is extremely difficult to remove every single mussel from an infested watercraft. Combining the methods ensures that the decontamination process is removing mussels and killing them; hence reducing the biological risk.

When using pressurized and hot-water sprays as a form of decontamination is not feasible, other methods, such as chemical control can be used. It is recommended to use either Quat™ 128 or Quat™ 256 to decontaminate wildland firefighting equipment that may have been exposed to dreissenids. It is recommended to use 0.5% Quat™ 128 for 1 min or 0.25% Quat™ 256 for 1 min to decontaminate wildland firefighting equipment exposed to *D. rostriformis bugensis*. To decontaminate equipment exposed to *D.*

polymorpha, it is recommended to use 0.75% Quat™ 128 for 5 min or 0.25% Quat™ 256 for 5 min. These recommendations are made based on the results that showed a 100% mortality rate in all water treatment groups with the smallest chemical concentration and exposure time. When the species is unknown, use the most conservative recommendation of 0.75% Quat™ 128 for 5 min or 0.25% Quat™ 256 for at least 5 min. Chemical solutions may be used for up to ten days without losing potency, unless the solution has been diluted from repeated use. Keep the chemical solution covered to minimize evaporation and/or dilution and to keep unwanted debris out. To determine if the solution is at the correct strength, use "Quat Chek 1000" Test Papers, which function like Litmus paper tests (U.S. FS, 2014). Finally, do not dump treated water into any water source, or on areas where it can migrate into any water body, storm drain, or sensitive habitat (U.S. FS, 2014). It is not advised to dispose large quantities of Quat chemicals in municipal sewer systems, as the chemical may disrupt the system's operations. Quat™ 128 and Quat™ 256 should only be disposed of in accordance with the label and MSDS directions.

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