DETERMINING EFFECTIVE DECONTAMINATION METHODS FOR WATERCRAFT EXPOSED TO ZEBRA MUSSELS, *Dreissena polymorpha* (Pallas 1776), THAT DO NOT USE HOT WATER WITH HIGH PRESSURE SPRAY

ERIC ANTHONY DAVIS

Zebra mussel veligers under cross-polarized light

Occasional Paper No. 52
State University of New York
College at Oneonta
OCCASIONAL PAPERS PUBLISHED BY THE BIOLOGICAL FIELD STATION

No. 1. The diet and feeding habits of the terrestrial stage of the common newt, Notophthalmus viridescens (Raf.) M.C. MacNamara, April 1976
No. 2. The relationship of age, growth and food habits to the relative success of the whitefish (Coregonus clupeaformis) and the cisco (C. artedi) in Otsego Lake, New York. A.J. Newell, April 1976.
No. 34. Trout movements on Delaware River System tail-waters in New York State. Scott D. Stanton, September 2000.
No. 37 Plans for the programmatic use and management of the State University of New York College at Oneonta Biological Field Station upland natural resources, Willard N. Harman. May 2003.

Continued inside back cover
Annual Reports and Technical Reports published by the Biological Field Station are available at:
http://www.oneonta.edu/academics/biofld/publications.asp
DETERMINING EFFECTIVE DECONTAMINATION METHODS FOR WATERCRAFT EXPOSED TO ZEBRA MUSSELS, *Dreissena polymorpha* (Pallas 1776), THAT DO NOT USE HOT WATER WITH HIGH PRESSURE SPRAY

ERIC ANTHONY DAVIS

Biological Field Station, Cooperstown, New York

bfs.oneonta.edu

STATE UNIVERSITY COLLEGE AT ONEONTA
The information contained herein may not be reproduced without permission of the author(s) or the SUNY Oneonta Biological Field Station
**ABSTRACT**

The introduction of the zebra mussel into freshwater systems all over the globe has led to great ecological and economical losses. By filter feeding on phytoplankton, the mussels cause an increase in water clarity leading to changes in the macrophyte community and reduction in the number of prey items available for small fish by outcompeting zooplankton. The mussels’ attachment to substrate leads to alterations of the benthic invertebrate community and can encourage the growth of some benthic blue-green algae. When larval stage mussels, called veligers, are taken in by water treatment or power production facilities they can attach to the inside of the plumbing, leading to decreased efficiency of the pipes and potentially causing complete stoppages. The costs associated with prevention and management of mussels in the pipe works are then placed on the customers of those facilities. In order to limit the odds of zebra mussels being transported to bodies of water that are mussel-free, the development of decontamination protocols has become very important.

The ability of gear and equipment to become encrusted or entangled with zebra mussels could allow for an expedited invasion by placing sexually mature individuals in close proximity to one another, enhancing the odds of reproductive success. Microscopic veligers can be transported in small amounts of water without being easily noticed. While studies have indicated that hot water/high pressure wash stations are effective at decontaminating watercraft, it is not economically feasible to create wash stations at every public boat launch. Watercraft owners may also resist using a wash station if the area is busy and simply go home without decontaminating their boat. To help alleviate this problem, methods of decontamination that could be performed by boat owners at locations other than a boat launch were examined. The methods included the use of chemical treatments on adult mussels and veliger larvae, using a commercial carwash to remove attached mussels, and using a garden hose to flush veligers from the livewell of a boat.

The toxicity of multiple chemicals to adult mussels and veliger larvae was examined to determine if they should be suggested as possible decontamination options for watercraft owners to use. The chemicals tested were selected for based upon their relative ease to obtain and their cost. Distilled white vinegar was the most effective chemical tested on adult mussels, while potassium chloride was the second most effective chemical. Next, sodium chloride and iodized table salt were equally effective and water softener salt was the least effective. The use of chemical treatments on the exterior of watercraft where mussels may be attached is unlikely due to the required exposure time to cause mortality but their application to areas that can hold the chemical solutions such as livewells, bilges, and anchor boxes would allow for exposure times that cause complete mortality. The submersion of gear and equipment into a chemical solution bath would also be possible. Virkon Aquatic and distilled white vinegar caused complete
mortality of veligers in 2 and 10 minutes, respectively. Potassium chloride was found to be more effective than sodium chloride at causing mortality to veligers.

Watercraft left in a body of water for extended periods of time have the capability of having veligers settle and go through metamorphosis to become attached mussels to the exterior of the boat. Mussels that are attached to a watercraft can shed their byssal threads and fall to the benthic environment when introduced to a lake. In order to stop the spread of mussels attached to the hull of a watercraft, the use of a pressure washer similar to those used at commercial carwashes to remove attached mussels was examined. It was found that attached mussels could be physically removed using a pressure washer similar to a commercial carwash but the time required to remove mussels from an entire watercraft could be longer than an average boat owner is willing to spend washing their boat.

The residual water left in a watercraft when it is trailered can contain veligers that could be released into the next body of water in which the boat is used when the water is removed by the bilge pump or when filling the livewell. The ability to flush the livewell of a boat with a garden hose was examined. It was found that a 5 minute flushing did not remove 100% of veligers from the livewell, but a 3 minute flushing removed more than 90% of veligers. Livewell flushing may greatly reduce the number of veligers present in the livewell, making invasions less likely compared to not flushing the livewell.
ACKNOWLEDGEMENTS

The completion of the research and this document would not have been possible without Dr. David Wong and his contagious desire to find the best way to prevent the spread of dreissenid mussels. His work ethic is second to none and his willingness to offer insight and advice has greatly helped me in the completion of my degree. Dr. Willard Harman has also been extremely helpful by providing insight from a different line of thinking and making sure any supplies or equipment that was available for use was claimed for the project. Dr. Jeffrey Heilveil and Dr. Florian Reyda have aided by reviewing my drafts and providing comments. Matt Albright and Holly Waterfield at the Biological Field Station helped with laboratory space use and research design input.

The funding for the research described in this document came from the Environmental Protection Fund which is administered by the New York State Department of Environmental Conservation. Without the continued funding provided by the excise taxes on hunting and fishing goods from the Pittman-Robertson and Dingle-Johnson Acts, the conservation of fish and wildlife resources and the research that aids in the best management possible would be severely hampered.

Finally, I would like to thank my family and friends for supporting my pursuit of an advanced degree to aid in my search for a career in the natural resources field. My fiancée Myranda has been extremely supportive and understanding of the required long days and late nights that come with biological research.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER 1: INTRODUCTION</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAPTER 2: DISTILLED WHITE VINEGAR (5% ACETIC ACID) AS A POTENTIAL DECONTAMINATION METHOD FOR ADULT ZEBRA MUSSELS</td>
<td>10</td>
</tr>
<tr>
<td>CHAPTER 3: COMPARISON OF THREE SODIUM CHLORIDE CHEMICAL TREATMENTS FOR ADULT ZEBRA MUSSEL DECONTAMINATION</td>
<td>20</td>
</tr>
<tr>
<td>CHAPTER 4: TOXICITY OF POTASSIUM CHLORIDE COMPARED TO SODIUM CHLORIDE FOR DECONTAMINATION OF ZEBRA MUSSELS</td>
<td>34</td>
</tr>
<tr>
<td>CHAPTER 5: TWO CHEMICALS FOR RAPID ZEBRA MUSSEL DECONTAMINATION</td>
<td>49</td>
</tr>
<tr>
<td>CHAPTER 6: ABILITY TO USE A COMMERCIAL CARWASH TO REMOVE ATTACHED MUSSELS</td>
<td>58</td>
</tr>
<tr>
<td>CHAPTER 7: LIVEWELL FLUSHING TO REMOVE MUSSEL VELIGERS</td>
<td>64</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>70</td>
</tr>
</tbody>
</table>
The introduction of non-native species has become one of the leading causes of declines in native organism diversity (Pysek and Richardson 2010). While this is especially true for aquatic invasive species (AIS), AIS not only cause ecosystem alterations but can inflict significant economic damages as well. In the United States, management of AIS cost more than $7 billion annually (Pimentel et al. 2005). This number was estimated in 2005, so it can be assumed that the costs associated with AIS through damage and prevention has grown since then due to inflation and additional invasions. One species that causes both ecological and economic damage in the United States is the zebra mussel, *Dreissena polymorpha* (Pallas 1771). The zebra mussel’s native range includes the Black, Caspian, and Azov seas (Benson et al. 2014). They are prolific spawners and can inhabit a wide range of habitats within water systems. A single female zebra mussel can produce up to 1 million eggs during spawning (Keller et al. 2007). Typically, zebra mussels start spawning when the water temperature reaches 12° Celsius (Mackie and Claudi 2010). Only one year after being first discovered, zebra mussels in Oneida Lake, New York, USA were found at densities of 44,000 mussels per square meter (Zhu et al. 2006).

The first discovery of a zebra mussel in North America came in 1986 in Lake Erie (Carlton 2008). The species quickly spread throughout the northeast United States, presumably via trailered watercraft (Johnson et al. 2001). Although adult mussels are easy to see and remove from a trailered watercraft, the larval stages can easily be spread in small amounts of residual water in the watercraft or trailer. Other vehicles that are in contact with water while moving may also serve as transport vectors for veligers (Johnson et al. 2001). These include wildland firefighting equipment, seaplanes, and fish hatchery haul trucks (Johnson et al. 2001). Veligers, the larval life stage of mussels, can range in size from 70 µm to just under 300 µm (Mackie and Claudi 2010). The veligers are planktonic, meaning they float freely in the water column while they develop. There are four major developmental stages of the veliger, they are: trophophore, D-shape or straight-hinged, umbonal, and pediveliger (See Figure 1). The average time to settlement for dreissenids is 18-30 days after fertilization, depending on the water conditions and temperature (Mackie and Claudi 2010). Once they reach the pediveliger size, larvae settle onto the substrate and metamorphose into juvenile mussels. While they are small, veligers are very hardy and resilient. Quagga mussel (*Dreissena bugensis*) veligers taken from Lake Mead in Nevada were shown to live in an average amount of water left in a trailered watercraft for up to 27 days during autumnal conditions (Choi et al. 2013). This time frame would make it possible to transport a vessel that has mussel veligers in its residual water over long distances before launching the vessel and infecting a new body of water.
Members of Dreissenidae produce byssal threads. These threads are secreted from the byssal gland, located within the muscular foot of the mussel (Mackie and Claudi 2010). The byssal threads protrude from the dorsal side of the mussel and look like hairs. They are what the mussels use to attach to hard surfaces, including rocks, pipes, and undersides of ships. The ability to attach to and live on hard surfaces is what makes the dreissenid mussel such a devastating organism in freshwater systems. Native freshwater mussels (family Unionidae) in North America typically inhabit areas with soft substrate where they bury themselves so that only their siphons are above the benthic surface. This would mean that there is a relatively low level of water fluctuation in that area because sedimentation has occurred over a very long period of time. The use of byssal threads for attachment allows the Dreissenids to attach to substrate in areas with a greater amount of water fluctuation or flow (up to 2 m/s [Mackie and Claudi 2010]). This can be advantageous because the zebra mussel is a suspension filter feeder. The increase in water flow would mean that food particles would be moved around more in the water allowing them to have the food particles brought to them by the water current. This would also allow any waste products excreted by the mussel to be taken away via water currents before the mussel could potentially take in self-contaminated water. The angle of the zebra mussel shell may help
the mussel maintain a certain level of filtration by deflecting water from directly entering the incurrent siphon at a rate too fast for the mussel to handle (Kauffman 1969). Dreissenids don’t only colonize in areas of hard substrate, however. In areas of soft or fine substrate, the mussels will produce druces. A druce is a ball of mussels that are attached to either the byssal thread of other mussels or to the shell of another mussel. Typically, as a druce is slowly taken apart, the inner most mussel will have a small piece of sediment in its byssal threads, such as a pebble or grain of sand. Zebra mussels have also been shown to prefer colonizing the shells of native mussels over other substrates (Riccardi et al. 1996).

Dreissenid biofouling commonly leads to the clogging of intake pipes and screens at water treatment and power-producing facilities. Dreissenid biofouling can even result in a complete stoppage of flow within a pipe system. The resulting reduction in efficiency in these facilities can lead to various problems and be expensive to manage and control. Connelly et al. (2007) reported the costs of zebra mussels from 1989-2004 to drinking water and power plant facilities at over $267 million, with an average cost per year of $571,009. Most of the cost reported was for mussel prevention; about one-third of total costs (Connelly et al. 2007). The next largest cost was lost production or revenue followed by chemical treatments, planning and designing, and retrofitting systems (Connelly et al. 2007). As expected, larger facilities had larger costs associated with zebra mussels, with facilities that produce more than 10 million gallons of water per day having costs 3-5 times that of facilities that produce fewer than 10 million gallons per day (Connelly et al. 2007). The facility passes the cost onto the customer or the taxpayer depending on whether the facility produces power or drinking water, and who owns the facility. One positive to the study was that the cost of zebra mussels to facilities was lower at the end of the study period than at the beginning (Connelly et al. 2007). This was attributed to early lack of knowledge on treatment options and the fact that facility operators may change plants and bring with them previous knowledge that significantly shortens the learning curve at facilities with new zebra mussel infestations (Connelly et al. 2007). Another form of cost to water facilities is an increase in pipe corrosion and flow alteration from byssal threads that are left attached to the pipes. The area around the attachment point of a byssal thread can become inhabited by bacteria that produce acidic byproducts from anaerobic respiration that can impact pipe integrity (Mackie and Claudi 2012).

Of the greater than $7 billion impact that AIS organisms cause in the United States, Pimentel et al. estimated that $1 billion is due to dreissenid mussels (2005). This figure does not include the environmental changes to beaches, boating, or fishing (Pimentel et al. 2005). In the state of New York, the dreissenid mussels cost an estimated $12.5 million in damages and control costs in the New York canal and Hudson River systems (Pimentel 2005). The $12.5
million is broken down into several categories. They are: tourism ($0.5 million), electric industry ($10 million), commercial fishery ($0.5 million), sport fishery ($1.0 million), and boating ($0.5 million) [Pimentel 2005]. The New York State Canal System is 524 miles long and it connects Lake Erie and Lake Ontario, the Finger Lakes, the Mohawk River, and Lake Champlain (Pimentel 2005). This encompasses most of the major waterways in New York and can most likely explain how dreissenid mussels were able to spread so rapidly throughout the state. It is also estimated that the cost of dreissenids is over $500 million in the Great Lakes basin (Pimentel 2005). The total cost is broken into several categories similar to those of the canal and Hudson River system. For the Great Lakes basin, the costs are: tourism ($0.5 million), electric industry ($480 million), commercial fishery ($13 million), sport fishery ($5 million), boating ($0.5 million), and transport ($1 million) [Pimentel 2005]. The cost to tourism is mainly because of the mussels’ colonization of beaches where people swim and wade, leading people to cut their feet. People who cut their feet on mussel shells will have negative memories of visiting certain locations that could cause them to shy away from returning in the future. The major cause of the cost to the fisheries and transport industry was hull fouling, leading to increased drag in the water and the need to clean gear and boats to remove mussels (Pimentel 2005).

The overall impact of zebra mussels on an ecosystem itself can cause economic damages. Studies have indicated a shift in macroinvertebrate communities in zebra mussel beds (Botts et al. 1996, Horvath et al. 1999, Stewart et al. 1996), alterations in macrophyte communities following zebra mussel infestations (Zhu et al. 2006, Zhu et al. 2007), changes in the plankton communities (Hecky et al. 2004, Wong and Levington 2005), and a modification to fish communities (Hoyle et al. 2008, Strayer et al. 2004, Watzin et al. 2008). Understanding how mussels affect ecosystems is a valuable tool and can show how they ultimately lead to economic losses via negative influences on other organisms.

Dreissenids are known for their ability to filter phytoplankton as the majority of their diet. This is what typically causes the increase in water transparency, as measured by Secchi depth, and the overall increase in the photic zone (Zhu et al. 2006). Zooplankton are an important group of organisms within aquatic ecosystems because they commonly are the link between primary production and consumption by small fishes. It is commonly thought that the overall decrease in phytoplankton leads to the decrease in zooplankton due to a lack of food availability. However, it has been shown that zebra mussels are able to feed on zooplankton as well as phytoplankton. Wong and Levington (2005) found that zebra mussels were able to consume two rotifer species that were common in the Hudson River prior to mussel invasion. This shows that zebra mussels not only compete with zooplankton for resources but they also can directly prey upon them. Another potential effect that mussel feeding may have on the planktonic community is an increase in blue-green algae, or cyanobacteria. Selective feeding on green algae by dreissenids leaves cyanobacteria in the water at a higher proportion than they usually occur
One such cyanobacterium is *Mycrocystis*, which can produce toxins that harm human skin, liver, and nervous systems (GLERL, 2014). Those toxins can be retained in the water even after the algae are dead. This is what caused the Toledo, Ohio, drinking water system to be shut down in August 2014. Nearly 500,000 people in and around Toledo were without potable water for days. The Associated Press reported that the state of Ohio spent over $187,000 on bottled water that was provided to Toledo residents and that Toledo has spent millions of dollars to treat its water to remove the toxins (Associated Press, 2014). Another alga that benefits from zebra mussel invasions is *Cladophora*, a benthic alga (Hecky et al. 2004). The increase in benthic structure, as seen by Horvath et al. 1999, allows for attachment by *Cladophora*. Also, the increase in water clarity (Zhu et al. 2006) allows greater light to penetrate to the benthos where *Cladophora* grows. A rise in carbon dioxide concentration caused by mussel respiration may also contribute to algal growth (Hecky et al. 2004). Excessive benthic algal growth can deter macrophyte growth by using the available resources, and it can lead to anoxia in deep water by adding large amounts of organic matter when algae die and sink into the hypolimnion to be broken down (Hecky et al. 2004).

In areas where zebra mussels become prevalent, the macroinvertebrate community changes. Overall invertebrate abundance increased in a lake-outlet stream in areas where simulated mussel beds were placed (Horvath et al. 1999). This growth in population did not differ between living mussel beds and beds comprised only of empty shells, indicating that the increase in epibenthic structure draws more invertebrates, most likely to seek shelter from predators. Other studies have however, shown that living mussel beds attract more invertebrates than dead mussel beds (Botts et al. 1996, Stewart et al. 1998). This difference was hypothesized to be due to the flowing water in the stream (Horvath et al. 1999). The flowing water likely swept away any organic matter, such as mussel feces, that may attract organisms that feed on detritus (Horvath et al. 1999). The majority of macroinvertebrates in the lake-outlet stream were filter feeders, indicating that they were not drawn to the shells due to deposits of organic matter. While invertebrate abundance may increase, the invertebrate community structure most likely changes due to zebra mussel presence.

As a result of the filter feeding of dreissenid mussels, the macrophyte community in water bodies has also been shown to change. In Oneida Lake, the average Secchi depth increased from 2.6 m prior to mussel inhabitation to 3.5 m (Zhu et al. 2006). As a function of increased Secchi depth, the amount of suitable habitat for submerged macrophytes increased from 90 km² before to 111 km² after the mussel invasion (Zhu et al. 2006). As a result of this increase in the photic zone, the macrophyte community shifted from shade-tolerant to species that can tolerate a range of light from 1975 to 2002 (Zhu et al. 2006). The maximum depth at which macrophytes were found increased from 3.0 m to 5.1 m while the species richness of macrophytes also increased from 8 to 12 species (Zhu et al. 2006). The increase in macrophytes can also affect the
macroinvertebrate, plankton, and fish communities. Many species rely on macrophytes for at least some part of their life cycle, whether for holding eggs or providing shelter for larval stages. Zebra mussels can also foul plants by attaching directly to macrophytes, potentially causing harm to the plant by weighing down the plant or by shading the plant and preventing photosynthesis from occurring (Zhu et al. 2007). It has been observed that zebra mussels settle on submersed aquatic plants or the underside of substrates (Ackerman et al. 1994). In Oneida Lake, Eurasian watermilfoil (Myriophyllum spicatum) plants had a decreased quantum yield, chlorophyll $a$ concentration, and total chlorophyll concentration when zebra mussels were attached to the stem and leaves (Zhu et al. 2007); however, eelgrass (Vallisneria americana) plants did not have any significant differences in quantum yield, chlorophyll $a$ concentration, and total chlorophyll concentration when zebra mussels were attached to them (Zhu et al. 2007). It was suggested that the difference in how the macrophytes were affected could be based on how the mussels attach to them. Milfoil has skinny stems and leaves that grow vertically and horizontally, allowing for easy attachment by settling mussels, while eelgrass grows vertically and closer to shore where the wave action may be too strong for mussels (Zhu et al. 2007). It can therefore be said that the effects of macrophyte fouling from dreissenid mussels are species specific due to plant morphology.

The impacts of zebra mussels on fish communities depend greatly upon the ecosystem that is invaded and the fish species that are present. Most changes to fish communities come from indirect changes to the ecosystem from the filter feeding of the mussels (Strayer et al. 2004). There are three indirect pathways in which filter feeding bivalves can impact fish: the impact on phytoplankton and edible consumers, the impact on benthic invertebrates, and the impact of increased littoral production (Strayer et al. 2004). The loss of phytoplankton and edible consumers is often detrimental to open water fishes that feed on phytoplankton and their primary consumers or fish that rely on lower water clarity to stay hidden from predators. Hoyle et al. (2008) showed that lake whitefish (Coregonus clupeaformis) and walleye (Sander vitreus) both have had overall declines in age class structure in eastern Lake Ontario. The decline in whitefish was primarily due to the loss of a key prey item and its replacement with lower quality foods (Hoyle et al. 2008). Following the decline, the remaining whitefish had a lower body condition and slower growth rate (Hoyle et al. 2008). The decline in walleye was attributed to the overall increase in water clarity resulting in poor recruitment of early life stages of fish and an overall shift to a consistently smaller size (Hoyle et al. 2008). In the Hudson River, open water species of fish moved downstream away from the area of greatest mussel establishment and had a decrease in growth rate (Strayer et al. 2004). The increase in benthic habitat and biodeposits that attract some foraging benthic invertebrates due to mussel beds should benefit fish that feed upon those invertebrates (Strayer et al. 2004). Littoral fish species in the Hudson River shifted upriver to the area of mussel infestation and had an increase in growth rate (Strayer et al. 2004). The impact of mussels on the production of the littoral zone should benefit fish species that feed upon
the invertebrates that live on or in the macrophyte stands that are able to expand (Strayer et al. 2004). In addition to the indirect effects of mussels on fish communities, there are some direct effects. In Lake Champlain, freshwater drum (*Aplodinotus grunniens*) and pumpkinseed (*Lepomis gibbosus*) fed upon zebra mussels heavily, while yellow perch (*Perca flavescens*) and rock bass (*Ambloplites rupestris*) fed on zebra mussels lightly (Watzin et al. 2008). Both drum and pumpkinseeds would pick up mussels and chew them, then expel the shell fragments while ingesting the soft tissue of the mussel (Watzin et al. 2008). The perch and rock bass would ingest small mussels whole, and the soft tissue would usually still be contained inside the shell in the fish’s intestine (Watzin et al. 2008). This could indicate that some species of naturalized fishes are shifting their diets to include zebra mussels.

With all of the effects to ecosystems that are attributed to the introduction of the invasive dreissenid mussels, it is relatively easy to see how ecological impacts can be turned into economic impacts. In 2011, fisherman in the state of New York contributed nearly $4.5 billion to the state’s economy (New York State Department of Environmental Conservation, 2014). Fisherman contribute not only license fees, which fund the state’s management of fish and wildlife populations, but they also spend money on gas, bait, food, drinks, and other miscellaneous gear. Some people take vacations to go fishing, spending money on hotels and possibly guides. With a decrease in fish size and population size, some people may start to shy away from fishing because they don’t catch that many fish or the fish they catch might be small. With increased water clarity, visually oriented fish may be able to recognize and avoid fishing gear, allowing them to avoid capture.

Preventing the continued spread of AIS is also a concern for some recreational boaters. If disinfection protocols are too time consuming or costly, some people may stop using their watercraft and give up fishing completely. Similar concerns may trouble waterfowl hunters who rely on watercraft to pursue ducks and geese. In the western United States, many agencies have begun watercraft interception programs to keep dreissenid mussels from spreading to uninfected water bodies (Zook and Phillips 2015). These programs involve watercraft inspection and decontamination. The United States Bureau of Reclamation has approved numerous forms of decontamination (DiVittorio et al. 2012). They include chemical treatment, heat, hot water/high pressure washing, freezing, physical removal, and desiccation (DiVittorio et al. 2012). Of the approved decontamination methods, hot water spray washing is the more commonly used method by most agencies (Comeau et al. 2011, DiVittorio 2015, Zook and Phillips 2015). Hot water spray combines the use of heat with the mechanical removal of mussel via the high pressure from the spray (DiVittorio et al. 2012). Adult mussels can be killed by exposure to below-freezing temperature; however clusters of adults are more resilient and can take almost three times longer to reach complete mortality at the same temperature (McMahon et al. 1993). The use of heat as a decontamination method consists of using steam or boiling water to raise the
surface temperature to 140\(^{\circ}\) F for between 5 and 30 seconds (Comeau et al. 2011, DiVittorio et al. 2012, respectively). Physical decontamination involves crushing adult mussels and is very time and labor intensive (DiVittorio et al. 2012). Desiccation is the cheapest and easiest way to prevent the spread of mussels; however, the amount of time needed to completely dry out a watercraft varies on the climate and the season. While during the summer months, the time required to dry a boat can be short, the time needed in the spring or autumn may be significantly longer. In cooler, more humid climates mussels may survive up to 40 days out of water (DiVittorio et al. 2012). Chemical treatments can be more difficult to use because some chemicals can be toxic to non-target organisms or they can produce harmful byproducts (Watters et al. 2013). It also may require a well-developed plan to handle the chemicals after treating watercraft (DiVittorio et al. 2012).

There are some chemicals that are recommended for the decontamination of field equipment or aquaculture practices that are used to control the spread of dreissenid mussels (Waller et al. 1996, Pucherelli et al. 2014). However, some of the recommended treatments require the use of formalin (Waller et al. 1996). Formalin has been shown to have carcinogenic properties and therefore, can cause health concerns when people are repeatedly exposed to it. The use of undiluted white vinegar is recommended by the United States Bureau of Reclamation for the treatment of veligers but Bureau did not identify how it came to this conclusion (DiVittorio et al. 2012). The chemicals tested in many studies are high purity lab-grade chemicals that can be expensive; however, there are household chemicals that are very similar (e.g., commercial table salt versus lab-grade sodium chloride). If mortality of adult mussels and veligers could be achieved in a reasonable time frame using household chemicals, decontamination of exposed watercraft and gear may be a possibility at a low cost. This could be especially beneficial at remote, seldom-used, boat launches instead of building a hot water spray station. Five chemicals were examined to determine their toxicity to adult zebra mussels. They were distilled white vinegar, sodium chloride, iodized table salt, water softener salt, and potassium chloride. The toxicity of distilled white vinegar, sodium chloride, potassium chloride, and Virkon Aquatic to zebra mussel veligers was also examined.

The search for an easy method continues in an attempt to increase the likelihood that recreational watercraft owners decontaminate their watercraft. While there may be multiple ways to disinfect a watercraft, the general public may not be keen on all of them. Some decontamination methods require extended periods of time that boat owners may not be willing to wait before using their vessel again. Others may require multiple steps such as creating a chemical solution, applying the solution, and then recapturing the chemical solution following the decontamination period. Complex decontamination protocols could also decrease the willingness of watercraft owners to adhere to those protocols.
While the exterior of a vessel is often the focus of decontamination methods, the remaining water in a watercraft’s bilge and livewell is often of concern as it can harbor the microscopic veligers. The introduction of veligers via the bilge pump or livewell pump from the watercraft to the next body of water where the watercraft is used often considered how new infestations occur (Johnson et al. 2001). The ability to successfully flush the remaining veligers out of a watercraft bilge or livewell after it has been removed from the water would result in fewer zebra mussel infestations. A garden hose equivalent was used to examine the effectiveness of flushing the livewell of a watercraft to remove veliger larvae.

The transportation of physically attached adult mussels could occur in some locations and can result in a faster infestation if multiple adults fall off from a fouled boat and remain close to each other to allow external fertilization. Most agencies recommend hot water spray as the decontamination method of choice for watercraft that are docked through spawning. The cost to construct a hot water spray decontamination station can be expensive (i.e. $30,000 [Jensen, 2009]). Combined with the number of public boat launches in a state with a large number of lakes, such as New York, the total cost to implement hot water wash stations statewide could be astronomical. This does not include the cost associated with installing running water and electricity required to operate a hot water spray station as well as the salary for someone to operate the station to ensure the correct watercraft disinfection protocol is followed. For these reasons, the use of a pressure washer with a similar pressure to that of a commercial car wash will be tested to determine the feasibility of removing attached adult mussels.
CHAPTER 2: DISTILLED WHITE VINEGAR (5% ACETIC ACID) AS A POTENTIAL DECONTAMINATION METHOD FOR ADULT ZEBRA MUSSELS

Abstract

The spread of zebra mussels into new bodies of water is of great concern in the United States due to their economic and ecological costs. Some government agencies suggest the use of vinegar as a decontamination option but do not provide data to explain this decision. This study examined the toxicity of distilled white vinegar on adult zebra mussels at varying concentrations and exposure times. All tested concentrations (25, 50, 75, and 100%) caused complete mortality within four hours. These results indicate that distilled white vinegar can be used for the decontamination of adult zebra mussels. Key words: zebra mussels, decontamination, vinegar, acute toxicity.

Introduction

The introduction of non-native species has become one of the leading causes of native organism diversity declines (Pysek and Richardson 2010). Aquatic invasive species (AIS) are among some of the more commonly known examples. However, AIS are not only a cause of ecosystem alterations but can inflict significant economic damages as well (United States Army Corps of Engineers 2002). In the United States, AIS cost more than $7 billion annually (Pimentel et al. 2005). One AIS of great concern is the zebra mussel, Dreissena polymorpha (Pallas, 1771). Zebra mussels alone cost an estimated $1 billion in the United States (Pimentel et al. 2005). In the New York State Canal and Hudson River Systems, zebra mussels cost an estimated $12.5 million in control and management (Pimentel 2005). The ability of zebra mussels to colonize hard substrates using their byssal threads is what makes them such a problem. Biofouling occurs in water intake systems, such as drinking water and power-producing facilities, as mussels attach to the interior of pipes and other infrastructure. In response to a survey, facility managers reported a total cost of over $267 million due to zebra mussels from 1989–2004 (Connelly et al. 2007). The spread of zebra mussels across much of the northeast and upper Midwest of the United States from the Great Lakes, where they were first discovered in the North America, is most likely from overland transport by trailered watercraft (Johnson et al. 2001). In the western United States, many agencies have begun watercraft interception programs to keep dreissenid mussels from spreading to uninfected water bodies (Zook and Phillips 2012). These programs involve watercraft inspection and decontamination. The United States Bureau of Reclamation has approved numerous forms of decontamination (DiVittorio et al. 2012). They include hot water/high pressure washing, heat, freezing, physical removal, desiccation, and chemical treatment (DiVittorio et al. 2012). Chemical treatments can be one of the more difficult methods

to use because some chemicals can be toxic to non-target organisms or they can produce harmful byproducts (Watters 2011). It also may require a well-developed plan to handle the chemicals after treating watercraft (DiVittorio et al. 2012). However, chemical treatments may provide an option for remote, low use boat launches if the chemical used is in small quantities and can be easily contained for proper disposal. A low cost chemical that is effective in small doses would allow for maximum decontamination for minimal cost. The use of undiluted white vinegar is one chemical treatment recommended by the United States Bureau of Reclamation for the treatment of veligers, the larval life stage of zebra mussels, but they do not indicate any studies that were used to come to this conclusion (DiVittorio et al. 2012). White vinegar is a common chemical found in many households and can be obtained fairly easily at a relatively low price. Our hypothesis was that distilled white vinegar is effective in killing adult zebra mussels. The goal of this study was to investigate the lethality of distilled white vinegar to adult mussels. The data generated by this study will be used to design a test of distilled white vinegar toxicity to mussel veligers during the upcoming spawning season.

Methods

**Adult collection and preparation**

Rocks colonized by adult mussels were collected at the State University of New York College at Oneonta Biological Field Station (BFS) Thayer Boathouse on Otsego Lake, New York in water around 1m in depth using a variety of clam rakes. The rocks were brought back to the lab in trays and coolers. Mussels were removed from the rocks using a paint scraper (similar to Costa et al. 2008) and were placed into a small tray with a constant flow of fresh Otsego Lake water. Mussels bunched together via byssal threads were pulled apart and placed into the tray. Once all the mussels were in the tray, eleven mussels were selected at random and placed into a 800-micron mesh bag. Those with any physical damage were discarded. One hundred and fifty bags were filled with mussels. All extra individuals were placed into a separate bag. The bags were placed into a large aquarium (~50L) with a slow constant flow of lake water for at least 72 hours for the mussels acclimate to the bags and to determine any mortality due to handling and/or stress (similar to Comeau et al. 2011). The aquarium was lightly aerated with compressed air. After the 72-hour holding period, the mesh bags were removed one at a time and the mussels inside were examined for mortality. Any dead individuals were removed from the bags. If no mortality occurred, one mussel was selected at random, removed from the bag, and discarded. All bags had 10 individuals so that there were 30 individuals for every concentration-exposure period combination. The number of mortalities during the acclimation period was less than ten.

**Chemical treatment**

Fifteen glass tanks were rinsed with lake water and scrubbed with an abrasive sponge soaked in lake water. The tanks were then emptied, rinsed, and emptied again. Lake water was added to each tank in the volume needed to result in a total of 20L once the vinegar was added.
The four concentrations of vinegar used were 25, 50, 75, and 100% vinegar. There were three duplicates of each concentration as well as three control tanks with no chemicals. The distilled white vinegar used was a readily available commercial product (5% acetic acid, Great Value, Distributed by Wal-Mart Stores, Inc., Bentonville, AR). The vinegar was kept in a greenhouse at the BFS main lab overnight before testing. Airstones were added to each tank and they were lightly aerated with compressed air to ensure continued mixing of the treatment water. Once all tanks had been filled, ten bags of mussels were hung from a wooden dowel in each aquarium. Each bag represented an exposure time period (0, 1, 2, 4, 6, 12, 24, 48, 72, and 96 h). After the bags in all tanks were immersed, the time 0 bags were then removed from each tank. Once bags were removed from the treatment tanks, they were labelled with their exposure time and hung from wooden dowels in two large holding tanks with continuously flowing water from Otsego Lake. This created an environment that was very close to the natural conditions. Mussels removed from the 0 and 25% vinegar treatment mussels were placed into one tank and the mussels in the 50, 75, and 100% vinegar solutions were placed into the other tank. One dowel held all the bags from an individual tank. Mussels were left in this tank for at least 48 hours for recovery. Mussels were examined for mortality between 48 and 72 hours after being removed from their chemical treatments. This was done because it has been shown that mussels that appear dead at the end of a chemical treatment can recover after being placed in clean water free of chemicals (Pucherelli et al. 2014). Mortality was determined by the mussel having gaping valves when removed from the mesh bag and placed on a paper towel. Mussels with a slight gap had a blunt probe placed into the valve gap and if the mussel closed it was considered alive. A mussel with no movement of the valves after probing was considered dead. Only mussels with a slight valve gap were probed, or when more than half of a sample group was fully gaping showing mortality. After mortality assessment, the shell length of each mussel was measured using a Mitutoyo Absolute digital caliper (Model Number: CD-6”CX, Mitutoyo Corporation, Kawasaki, Japan) and recorded along with the mortality status.

Water quality

An YSI (Model Number: 6820V2-M, YSI Incorporated, Yellow Springs, Ohio) multiparameter water quality sonde was used in each chemical treatment tank at times 0, 24, 48, 72, and 96 h. Prior to each measurement, the YSI was calibrated following standard operation protocols to ensure probe accuracy. All tanks of the same concentration were measured starting from the control tanks and increasing in concentration. The probe was rinsed with lake water between concentration levels. The water in the recovery/holding tanks was measured at 0, 24, 48, 72, and 96 h. The parameters measured were temperature, specific conductivity, pH, and dissolved oxygen as percentage and as mg/L. Measurements were taken from the chemical treatment tanks after mussels had been removed for the same exposure time. Measurements in the recovery tank were also taken after mussels of the same exposure time were added to the tank. This was to measure the maximum amount of chemicals being added at a time in the
holding tank, as the constant flow of fresh lake water would replace the water in the tank over time diluting the chemical.

Statistical analysis

All data was entered into a Microsoft Excel spreadsheet. Descriptive statistics were run on the shell length of all mussels. It was also used to determine the average mortality for each concentration at each time interval and to create a graph showing mortality at each interval. This was used to create the LD50 and LD99 with 95% confidence intervals for times 1 and 2 h with SAS® (Version 9.3 SAS Institute Inc., Cary, NC). The LD50 and LD99 values are the concentrations of vinegar that are required to produce 50% and 99% mussel mortality. A t-Test was performed to compare the mean length of dead and alive mussels. A one-way ANOVA was performed for the average values for each water quality parameter grouped by concentration over time. A t-Test was performed to compare the mean values of each group whenever the ANOVA was significant. The water quality parameters of the two holding tanks were compared using a t-Test. The level of significance was set at $\alpha = 0.05$.

Results

The mean temperatures of the experimental tanks did not differ by concentration for the duration of the experiment (p-value 0.876, Table 2.1). The mean dissolved oxygen did not differ between concentrations as either a percentage or in mg/L (p-values 0.354 and 0.807). Mean specific conductivity was significantly different between concentration levels (p-value <0.001). The mean square of variation between groups was 1.34 and was 0.01 for within groups. The mean pH was also different between concentration levels (p-value <0.001). Mean square of variation between groups was 26.35 while the mean square for within groups was 0.07. Each concentration was different from all other concentrations for specific conductivity and pH when compared with t-Tests (all p-values <0.001). The temperature of each holding tank was significantly different over the duration of the study (p-value 0.045). The specific conductivity of each tank was not different (p-value 0.115). Dissolved oxygen concentration as % was not different between tanks (p-value 0.116) and neither was dissolved oxygen as mg/L (p-value 0.106). The pH values were different between holding tanks (p-value 0.026). Mean shell length for the mussels was 13.29 mm with a standard deviation of 2.94 mm. This number was calculated using the lengths of all mussels in exposure periods of 48 hours and shorter. Mussels exposed to any vinegar treatment for longer than 48 hours were not able to be measured due to the vinegar’s effects to the mussels (Figure 2.1). There was a significant difference between the length of dead and alive mussels (p-value <0.0001). The mean length of dead mussels was 12.99 mm and the mean length of live mussels was 13.92 mm. All concentrations of vinegar led to complete mortality of mussels in less than 24 hours (Figure 2.2). The concentration to cause 100% mortality the fastest was the full strength vinegar, with a one hour exposure period. The 75% vinegar treatment led to complete mortality with a two hour exposure, and the 50% and
25% vinegar treatments caused complete mortality with a four hour exposure. All exposure periods longer than four hours also caused complete mortality. The control group had 8 total mortalities. Seven mortalities occurred at the 1 hour exposure (4, 3, and 0 in the three replicates, respectively) and one mortality at the 72 hour exposure. The LD50 and LD99 values were calculated in % vinegar in the treatment and were 37.78% and 220.89% for the one hour exposure period and 24.65% (17.49–29.86 [95% CI]) and 83.30% (62.04– 160.40 [95% CI]) for the two hour exposure period (Table 2.2).
Table 2.1. Average water quality parameters measured from recovery tanks and chemical solution tanks at various times during testing.

<table>
<thead>
<tr>
<th>Tank/Concentration (% vinegar)</th>
<th>Time (h)</th>
<th>Temperature (°C)</th>
<th>Specific Conductivity (µS/cm)</th>
<th>pH</th>
<th>Dissolved Oxygen (%)</th>
<th>Dissolved Oxygen (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery Tank 1</td>
<td>0</td>
<td>15.25</td>
<td>0.300</td>
<td>6.55</td>
<td>92.9</td>
<td>9.30</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>14.51</td>
<td>0.303</td>
<td>7.16</td>
<td>93.9</td>
<td>9.56</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>14.97</td>
<td>0.307</td>
<td>7.06</td>
<td>97.8</td>
<td>9.87</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>15.84</td>
<td>0.312</td>
<td>6.64</td>
<td>94.0</td>
<td>9.29</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>15.14</td>
<td>0.306</td>
<td>6.85</td>
<td>94.7</td>
<td>9.51</td>
</tr>
<tr>
<td>Recovery Tank 2</td>
<td>0</td>
<td>15.25</td>
<td>0.300</td>
<td>6.55</td>
<td>92.9</td>
<td>9.30</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>14.59</td>
<td>0.305</td>
<td>7.10</td>
<td>94.2</td>
<td>9.59</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>15.04</td>
<td>0.303</td>
<td>6.77</td>
<td>97.2</td>
<td>9.79</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>15.84</td>
<td>0.306</td>
<td>6.41</td>
<td>90.3</td>
<td>8.92</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>15.18</td>
<td>0.304</td>
<td>6.71</td>
<td>93.7</td>
<td>9.40</td>
</tr>
<tr>
<td>0%</td>
<td>0</td>
<td>17.97</td>
<td>0.309</td>
<td>8.54</td>
<td>94.7</td>
<td>8.97</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>17.27</td>
<td>0.306</td>
<td>7.25</td>
<td>87.4</td>
<td>8.38</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>17.83</td>
<td>0.307</td>
<td>7.19</td>
<td>84.0</td>
<td>7.96</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>18.94</td>
<td>0.308</td>
<td>7.21</td>
<td>89.3</td>
<td>8.29</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>18.00</td>
<td>0.308</td>
<td>7.55</td>
<td>88.9</td>
<td>8.40</td>
</tr>
<tr>
<td>25%</td>
<td>0</td>
<td>15.17</td>
<td>0.839</td>
<td>2.63</td>
<td>91.4</td>
<td>9.15</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>16.93</td>
<td>0.834</td>
<td>2.66</td>
<td>81.3</td>
<td>7.86</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>17.66</td>
<td>0.879</td>
<td>2.70</td>
<td>63.8</td>
<td>6.94</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>18.94</td>
<td>1.119</td>
<td>2.78</td>
<td>87.8</td>
<td>8.12</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>18.99</td>
<td>1.205</td>
<td>2.69</td>
<td>81.1</td>
<td>8.02</td>
</tr>
<tr>
<td>50%</td>
<td>0</td>
<td>12.85</td>
<td>1.163</td>
<td>2.32</td>
<td>83.2</td>
<td>8.77</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>16.51</td>
<td>1.139</td>
<td>2.42</td>
<td>78.8</td>
<td>7.68</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>17.68</td>
<td>1.326</td>
<td>2.54</td>
<td>77.7</td>
<td>7.36</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>18.97</td>
<td>1.378</td>
<td>2.54</td>
<td>91.6</td>
<td>8.37</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>19.02</td>
<td>1.392</td>
<td>2.46</td>
<td>82.8</td>
<td>8.05</td>
</tr>
<tr>
<td>75%</td>
<td>0</td>
<td>10.94</td>
<td>1.397</td>
<td>2.15</td>
<td>83.6</td>
<td>9.19</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>16.41</td>
<td>1.322</td>
<td>2.31</td>
<td>84.4</td>
<td>8.25</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>17.72</td>
<td>1.492</td>
<td>2.44</td>
<td>86.5</td>
<td>8.19</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>18.95</td>
<td>1.550</td>
<td>2.44</td>
<td>82.1</td>
<td>7.55</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>19.05</td>
<td>1.655</td>
<td>2.33</td>
<td>84.1</td>
<td>8.29</td>
</tr>
<tr>
<td>100%</td>
<td>0</td>
<td>8.92</td>
<td>1.589</td>
<td>2.03</td>
<td>82.5</td>
<td>8.83</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>16.63</td>
<td>1.475</td>
<td>2.24</td>
<td>82.2</td>
<td>7.97</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>17.00</td>
<td>1.593</td>
<td>2.33</td>
<td>77.5</td>
<td>7.33</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>19.10</td>
<td>1.668</td>
<td>2.34</td>
<td>90.7</td>
<td>8.34</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>19.10</td>
<td>1.715</td>
<td>2.23</td>
<td>83.2</td>
<td>8.12</td>
</tr>
</tbody>
</table>
Figure 2.1: Adult zebra mussels after exposure to 50% distilled white vinegar for 72 hours.
Figure 2.2. Average mortality (%) of adult zebra mussels (N=3 groups, each with 10 mussels) from Otsego Lake after exposure to distilled white vinegar of varying concentrations (% vinegar) in Fall 2014.

Table 2.2. Estimated LD$_{50}$ and LD$_{99}$ of distilled white vinegar concentrations (% vinegar) for adult zebra mussels vinegar with 95% confidence intervals for exposure durations of 1 and 2 hours and the sample dose(s) that caused 100% mortality during the experiment.

<table>
<thead>
<tr>
<th>Exposure Time (h)</th>
<th>LD$_{50}$ (95% CI)</th>
<th>LD$_{99}$ (95% CI)</th>
<th>SD$_{100}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37.78$^a$</td>
<td>220.89$^a$</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>24.65 (17.49-29.86)</td>
<td>83.30 (62.04-160.40)</td>
<td>75, 100</td>
</tr>
</tbody>
</table>

Note: $^a$ indicates that no 95% confidence interval was generated.
Discussion

The low mean square of variation values given for the pH and the specific conductivity within treatment groups indicates that the values did not vary greatly throughout the experiment. The source of variation was much greater between groups, as it was expected. The ratio of vinegar and water in the tanks differed by concentration, leading to differences in the amount of dissolved ions in the water when measured as specific conductivity. The difference in the amount of ions within each concentration also materialized in the pH readings. The greater the concentration of vinegar there was, the lower the pH value. The temperature at the beginning of the experiment was lower in the vinegar solutions compared to the recovery tanks, but the largest temperature difference was less than seven degrees. Adult mussels have been shown to handle temperature increases up to 10 degrees and decreases of up to 15 degrees with no mortality (Nichols 1992). The difference in the temperature of the holding tanks was found to be significant; however the difference between their means was 0.0375°C. The small sample size likely played a role in the difference being significant. The water coming from the lake was well oxygenated and had a consistent amount of dissolved ions in it. Neither tank came close to having anoxic conditions that would contribute to addition mortality not caused by the chemical treatments. The pH was different between the two recovery tanks and can be explained by how they were used. The first tank only received the mussels from the control and 25% vinegar treatments. The second tank received mussels from the 50, 75, and 100% vinegar treatments. When the mussels were added to the recovery tank, they usually had some amount of their treatment solution left in the mesh bag or on the surface of the mussels. So by adding three bags that came from tanks with significantly lower pH values, the second recovery tank pH value should decrease more than the first recovery tank. Also, the timing of measurement taking could have played a role. Water quality was measured directly after the bags of mussels were added to the recovery tanks. This would mean the lowest pH would be when the measurements were taken, but they would get diluted out as fresh lake water was flowing into the tank. It has been suggested that the pH tolerance range of adult zebra mussels is 6.5 (McCauley and Kott 1993) to 9.3 (Bowman and Bailey 1998). Claudi et al. (2012) found that a pH value of 6.9 caused about 40% mortality to adult mussels after an exposure of 10 weeks. The pH values of all vinegar concentrations in this study were well below 6.5, so mortality was expected. However, the speed of mortality caused by vinegar was not known and was of the greatest interest. The short exposure time causing complete mortality is a very positive indication for the use of distilled white vinegar as a decontamination method. It is recommended elsewhere that contaminated equipment be exposed to undiluted white vinegar for 20 minutes (DiVittorio et al. 2012). However, DiVittorio et al. (2012) do not indicate where they came up with this recommendation. This exposure period is intended for the treatment of veligers, not adults. It could be assumed that the time needed to kill veligers is shorter than needed to kill adults based on previous findings (Fisher et al. 1994). DiVittorio et al. (2012) do not recommend chemical treatments for
adults because they claim adults can close their valves for up to 10 days when exposed to chemicals. The current study indicates that adult mussels can be treated with chemicals to induce complete mortality in under 4 days. With all concentrations causing complete mortality in 4 hours, it can be assumed that an even lower concentration could be used as the minimum concentration to cause complete mortality with a 24 hour exposure. The four hour exposure with 25% vinegar solution does allow for a fast turn-around time without using as much vinegar. The use of such a strong acid solution may be problematic in certain applications where the materials that need to be disinfected could be harmed by the chemical treatment. A test under field conditions would also be beneficial to investigate the effects it may have on equipment. Further testing to examine lower vinegar concentration solutions could be beneficial so that there is a lower potential for damage. The likelihood of using the chemical treatment to disinfect the exterior of a watercraft is low because of the exposure time that is needed to induce complete mussel mortality. However, the use of a vinegar solution to disinfect equipment that is submersible such as lines, chains, and anchors is very feasible. The use of a vinegar solution to disinfect areas of a watercraft that can hold volumes of liquid such as livewells, bilges, and anchor boxes is also feasible because the chemical solution can be held in those places for the needed exposure period. Examining the exposure periods needed to cause complete mortality of veligers is of great value because they can be easily transported by trailered watercraft while not being visible to anyone inspecting the watercraft for AIS. The difference in the shell length of live and dead mussels was found to be significantly different. However, this difference should not be considered conclusive due to the fact that all shell lengths of mussels in exposure times of 72 and 96 hours were not included in the calculations. The addition of these shell lengths may have caused the mean lengths of dead and live mussels to be similar. All mussels used in this study were greater than 8mm in length, so they can be considered adults regardless of their mean lengths. Also, all mussels were killed regardless of length during exposures greater than 4 hours in all vinegar solutions. This study suggests that distilled white vinegar can be used at varying concentrations for the decontamination of adult zebra mussels. Vinegar is a common household chemical that is relatively inexpensive ($2.49 per gallon for this test) so it may have a positive impact on the decontamination effort put forth by the lay watercraft owner. An investigation into the impact vinegar solution have on equipment would be very informative to help determine its practical use as a decontamination chemical.
CHAPTER 3: COMPARISON OF THREE SODIUM CHLORIDE CHEMICAL TREATMENTS FOR ADULT ZEBRA MUSSEL DECONTAMINATION

Abstract

Chemical treatment for the control of the spread of zebra mussels in watercraft is typically focused on the early life stages of the mussel. Adult mussels may be spread via attachment or entangling to gear that is brought on board. Sodium chloride is a chemical that has been recommended for use during some aquacultural practices as a mussel disinfectant. The effectiveness of three sodium chloride-based salts (high-grade sodium chloride, iodized table salt, and water softener salt) was examined for their use as an adult zebra mussel decontamination solution. High-grade sodium chloride and iodized table salt both caused complete mortality at 30,000 mg/l in 24 h. Water softener salt caused complete mortality at the same concentration at 48 h. Iodized table salt caused complete mortality at a lower concentration faster than the laboratory-grade sodium chloride. On the basis of the results of this study, iodized table salt may be an acceptable alternative to high-grade sodium chloride for decontamination of zebra mussels, costing much less and leading to an increase in spread-prevention effectiveness.

Introduction

The zebra mussel *Dreissena polymorpha* (Pallas, 1776) is a bivalve native to the Black, Caspian, and Azov Seas in eastern Europe (Benson et al. 2014), which was discovered in North America in the 1980s. It spread rapidly throughout the northeast and upper mid-western United States, mostly through overland transport by trailered watercraft (Padilla et al. 1996, Johnson et al. 2001). The continued spread of the zebra mussel and a second dreissenid, the quagga mussel *Dreissena rostriformis bungensis* (Andrusov, 1897), into the western United States has led some government agencies to create inspection (Zook and Phillips 2015) and disinfection programs (DiVittorio 2015) to prevent their further spread. Chemical treatments are one of the several methods recommended for the decontamination for watercraft and equipment by the U.S. Bureau of Reclamation (DiVittorio et al. 2012). Chemical treatments are recommended for the treatment of larval stage mussels, not adults (DiVittorio et al. 2012). This is because adult mussels can detect chemicals, such as chlorine, in the water and close their valves to avoid additional exposure (Rajagopal et al. 2002). Recommended chemicals for zebra mussel decontamination are diluted household chlorine bleach, undiluted white vinegar, potassium permanganate solution, and quaternary ammonium solution (DiVittorio et al. 2012). The use of sodium chloride as a treatment for decontamination has been recommended for aquaculture practices where water-containing fish can be treated without causing mortality to the fish (Waller et al. 1996, Pucherelli et al. 2014). Both studies investigated the use of sodium chloride as a treatment for

---

veligers, not adults. This was because the water being used for aquaculture purposes can be filtered to keep adult mussels from entering haul trucks or other tanks (Waller et al. 1996). There are other situations where adult mussels could be transported overland, such as attachment to equipment or gear used during recreational boating or the collection of scientific data. This study looked to investigate the potential use of sodium chloride as a disinfectant for adult zebra mussels for recreational watercraft users. Our first hypothesis was that sodium chloride would cause 100% mortality of adult mussels within 96 h of exposure. The sodium chloride used in most laboratory testing is of high grade and is not the same as sodium chloride–based salts available to the average watercraft user. Therefore, the effectiveness of two sodium chloride–based salts (iodized table salt and water softener salt) was tested to compare their results to the high-grade sodium chloride results. Our second hypothesis was that the sodium chloride–based chemicals would work equally as well as the high-grade sodium chloride at causing mortality to adults.

Materials and Methods

Adult Collection and Preparation

Rocks colonized by adult mussels were collected in the fall of 2014 at the State University of New York College at Oneonta Biological Field Station Thayer Boathouse on Otsego Lake, NY, in water around 1 m depth using a variety of clam rakes. The rocks were brought back to the laboratory in trays and coolers. Mussels were removed from the rocks using a paint scraper (similar to Costa et al. 2008) and were placed into a small tray with a constant flow of fresh Otsego Lake water. Mussels bunches together via byssal threads were pulled apart and placed into the tray. Once all the mussels were in the tray, 11 mussels were selected at random and placed into a 800-mm-mesh bag. Those with any physical damage were discarded. A total of 150 bags were filled with mussels. All extra individuals were placed into a separate bag. The bags were placed into a large aquarium (~150 l) with a slow constant flow of lake water for at least 72 h for the mussels acclimate to the bags and to determine any mortality due to handling and/or stress (similar to Comeau et al. 2011). The aquarium was lightly aerated with compressed air. After the 72-hour holding period, the mesh bags were removed one at a time and the mussels inside were examined for mortality. If there was a dead individual, it was removed from the bag. If no mortality occurred, one mussel was selected at random and removed from the bag. If multiple mortalities occurred, the dead mussels were removed and live specimens from the extra bag of mussels were used to bring the total to 10 individuals per bag. The number of mortalities during the acclimation period was less than 10.

Chemical Treatment

A total of 15 glass tanks were rinsed with lake water and scrubbed with a sponge soaked in lake water. The tanks were then emptied, rinsed, and emptied again. To each tank, 20 l of
Otsego lake water was added. The needed amount of each chemical was measured on an electronic scale and then added to a tank. The four chemical concentrations used for the treatments were 500, 3,000, 10,000, and 30,000 mg/l; the highest concentration being the equivalent of seawater. Also, 10,000 mg/l was the concentration found to be effective on zebra mussel veligers and settlers by Waller et al. (1996). There were three replicates of each concentration as well as three control tanks with no chemicals. Once the salt was added to the water in a tank, the water was mixed with a wooden dowel until the chemical was completely dissolved. The tanks were prepared from weakest to strongest concentration (all 500 mg/l tanks were prepared, then the 3,000 mg/l tanks were prepared, etc.) and the dowel was rinsed with lake water between tanks. The chemicals that were tested were sodium chloride (NaCl; Amresco, Solon, OH), iodized table salt (Morton Salt, Inc., Chicago, IL), and sodium chloride–based water softener salt (Solar Extra Coarse Crystals; Culligan International Company, Rosemont, IL). Airstones were added to each tank and they were lightly aerated with compressed air to ensure continued mixing of the treatment water. Once all tanks had been mixed and all chemicals were dissolved, 10 bags of mussels were placed into each aquarium and hung from a wooden dowel. Each bag represented an exposure period (0, 1, 2, 4, 6, 12, 24, 48, 72, and 96 h). After all tanks had their bags immersed, the time 0 h bags were then removed from each tank. Once bags were removed from the treatment tanks, they were labeled with their exposure time and hung from wooden dowels in the large holding tank originally used for mussel assimilation to the bags. One dowel would hold all the bags from an individual tank. Mussels were left in this tank for at least 48 h for recovery. Mussels were examined for mortality between 48 and 72 h after being removed from their chemical treatments. This was done because it has been shown that mussels that appear dead at the end of a chemical treatment can recover after being placed in clean water free of chemicals (Pucherelli et al. 2014). Mortality was determined by the mussel having gaping valves when removed from the mesh bag and placed on a paper towel. Mussels with a slight gap had a blunt probe placed into the valve gap and if the mussel closed it was considered alive. A mussel with no movement of the valve after probing was considered dead. Only mussels with a slight valve gap were probed, or when more than half of a sample group was fully gaping showing mortality. After mortality assessment, the shell length of each mussel was measured using a Mitutoyo Absolute digital caliper (model number: CD-6”CX; Mitutoyo Corporation, Kawasaki, Japan) and recorded along with the mortality status. Between tests, tanks were emptied, scrubbed with an abrasive pad, rinsed with lake water at least two times, and then air-dried before the next round of testing was performed.

Water Quality
An YSI (model number: 6820V2-M; YSI Incorporated, Yellow Springs, OH) multiparameter water quality sonde was used in each chemical treatment tank at times 0, 24, 48, 72, and 96 h. Before each measurement, the YSI was calibrated following standard operation protocols to ensure probe accuracy. All tanks of the same concentration were measured starting
from the control tanks and increasing in concentration. The probe was rinsed with lake water between concentration levels. The water in the recovery/holding tank was measured at 0, 24, 48, 72, and 96 h. The parameters measured were temperature, specific conductivity, pH, and dissolved oxygen as percentage and as mg/l. Measurements were taken from the chemical treatment tanks after mussels had been removed for the same exposure time. Measurements in the recovery tank were also taken after mussels of the same exposure time were added to the tank. This was to measure the maximum amount of chemicals being added at a time in the holding tank, as the constant flow of fresh lake water would replace the water in the tank over time diluting the chemical.

Statistical Analysis

Descriptive statistics were run on the shell length of all mussels. They were also used to determine the average mortality for each concentration at each time interval and create a graph showing mortality at each interval. Mortality data were used to create the LD50 and LD99 with 95% confidence intervals (CI) for 4, 6, 12, and 24 h with SAS (version 9.3; SAS Institute Inc., Cary, NC). The LD50 and LD99 were the concentrations of chemical that would result in 50% and 99% mortality of mussels for the selected exposure time. It was determined that 24 h would be the longest time that would be considered a reasonable amount of time for a watercraft to be decontaminated with chemical treatments. An analysis of variance was used to compare the shell length of mussels that were killed and those that survived, as well as by concentration. Whenever any differences were found, a Student–Newman–Keuls test was performed to determine whether the difference was between the dead and live mussels, between treatment groups, or both. The level of significance was set at $\alpha = 0.05$.

Results

The water quality parameters (Table 3.1) in the treatment tanks and the holding tank all stayed within the range shown to be tolerated by zebra mussels in the literature during each experiment (Nichols 1992, McMahon 1996, Mackie & Claudi 2010, Claudi et al. 2012).

Sodium Chloride

The mean length of mussels in this study group was 13.98 mm with an SD of 2.96 mm. The mean length of the dead mussels was significantly different from the mean length of the live mussels ($F = 12.15, P < 0.0001$) when mussels in the control group were taken into account. The mean length of the control group was 15.03 mm compared with the other four concentrations (range 13.60–13.89 mm). The control group was removed and the mussels from the treatments were used to compare the mean lengths of the live and dead mussels. No difference was found with the control group removed ($F = 3.35, P = 0.0674$). Dead mussels’ average shell length was 13.41 mm and live mussels’ average shell length was 13.79 mm.
Table 3.1. Collection date, experiment start date, and average water quality parameters measured from the holding tank and experimental tanks (N=3 tanks per concentration level) at 24 hour intervals during each experiment in the fall of 2014.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Collection Date</th>
<th>Experiment Start Date</th>
<th>Tank/Concentration</th>
<th>Time (h)</th>
<th>Temperature (°C)</th>
<th>Specific Conductivity (mS/cm)</th>
<th>pH</th>
<th>Dissolved Oxygen (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High grade sodium chloride</td>
<td>16 October</td>
<td>19 October</td>
<td>Holding Tank</td>
<td>0</td>
<td>16.40</td>
<td>0.312</td>
<td>8.39</td>
<td>9.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>16.30</td>
<td>0.368</td>
<td>8.59</td>
<td>9.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>17.05</td>
<td>0.288</td>
<td>9.11</td>
<td>8.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>16.86</td>
<td>0.299</td>
<td>9.15</td>
<td>8.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96</td>
<td>16.67</td>
<td>0.309</td>
<td>9.18</td>
<td>8.97</td>
</tr>
<tr>
<td>0 mg/L</td>
<td>0</td>
<td>15.05</td>
<td></td>
<td>0</td>
<td>15.05</td>
<td>0.298</td>
<td>8.40</td>
<td>9.63</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>18.27</td>
<td></td>
<td></td>
<td>0.315</td>
<td>8.14</td>
<td>10.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>18.75</td>
<td></td>
<td></td>
<td>0.291</td>
<td>8.43</td>
<td>9.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>18.81</td>
<td></td>
<td></td>
<td>0.291</td>
<td>8.51</td>
<td>9.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>18.88</td>
<td></td>
<td></td>
<td>0.292</td>
<td>8.60</td>
<td>9.03</td>
<td></td>
</tr>
<tr>
<td>500 mg/L</td>
<td>0</td>
<td>15.19</td>
<td></td>
<td>0</td>
<td>15.19</td>
<td>1.253</td>
<td>8.09</td>
<td>9.52</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>18.16</td>
<td></td>
<td></td>
<td>1.303</td>
<td>8.14</td>
<td>10.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>18.55</td>
<td></td>
<td></td>
<td>1.210</td>
<td>8.22</td>
<td>9.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>18.67</td>
<td></td>
<td></td>
<td>1.214</td>
<td>8.27</td>
<td>9.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>18.79</td>
<td></td>
<td></td>
<td>1.219</td>
<td>8.33</td>
<td>9.02</td>
<td></td>
</tr>
<tr>
<td>3,000 mg/L</td>
<td>0</td>
<td>15.18</td>
<td></td>
<td>0</td>
<td>15.18</td>
<td>5.628</td>
<td>8.08</td>
<td>9.46</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>18.20</td>
<td></td>
<td></td>
<td>5.850</td>
<td>8.18</td>
<td>10.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>18.70</td>
<td></td>
<td></td>
<td>5.419</td>
<td>8.16</td>
<td>8.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>18.79</td>
<td></td>
<td></td>
<td>5.427</td>
<td>8.19</td>
<td>8.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>18.89</td>
<td></td>
<td></td>
<td>5.435</td>
<td>8.22</td>
<td>8.86</td>
<td></td>
</tr>
<tr>
<td>10,000 mg/L</td>
<td>0</td>
<td>15.18</td>
<td></td>
<td>0</td>
<td>15.18</td>
<td>16.813</td>
<td>7.91</td>
<td>9.20</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>18.26</td>
<td></td>
<td></td>
<td>17.477</td>
<td>8.07</td>
<td>9.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>18.81</td>
<td></td>
<td></td>
<td>16.197</td>
<td>8.08</td>
<td>8.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>18.89</td>
<td></td>
<td></td>
<td>16.230</td>
<td>8.09</td>
<td>8.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>18.98</td>
<td></td>
<td></td>
<td>16.263</td>
<td>8.11</td>
<td>8.38</td>
<td></td>
</tr>
<tr>
<td>30,000 mg/L</td>
<td>0</td>
<td>14.88</td>
<td></td>
<td>0</td>
<td>14.88</td>
<td>45.257</td>
<td>7.70</td>
<td>8.47</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>18.41</td>
<td></td>
<td></td>
<td>47.263</td>
<td>7.97</td>
<td>8.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>18.91</td>
<td></td>
<td></td>
<td>43.723</td>
<td>7.99</td>
<td>7.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>18.97</td>
<td></td>
<td></td>
<td>43.815</td>
<td>7.98</td>
<td>7.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>19.02</td>
<td></td>
<td></td>
<td>43.907</td>
<td>7.97</td>
<td>7.54</td>
<td></td>
</tr>
<tr>
<td>Iodized table salt</td>
<td>24 October</td>
<td>28 October</td>
<td>Holding Tank</td>
<td>0</td>
<td>15.85</td>
<td>0.339</td>
<td>8.37</td>
<td>9.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>18.30</td>
<td>0.336</td>
<td>9.01</td>
<td>8.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>17.14</td>
<td>0.323</td>
<td>8.96</td>
<td>9.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>16.55</td>
<td>0.316</td>
<td>8.93</td>
<td>9.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96</td>
<td>15.97</td>
<td>0.309</td>
<td>8.90</td>
<td>9.00</td>
</tr>
<tr>
<td>Treatment</td>
<td>Collection Date</td>
<td>Experiment Start Date</td>
<td>Tank/Concentration</td>
<td>Time (h)</td>
<td>Temperature (°C)</td>
<td>Specific Conductivity (mS/cm)</td>
<td>pH</td>
<td>Dissolved Oxygen (mg/L)</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------</td>
<td>-----------------------</td>
<td>--------------------</td>
<td>----------</td>
<td>------------------</td>
<td>--------------------------------</td>
<td>--------</td>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>17.16</td>
<td>0.307</td>
<td>8.22</td>
<td>9.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>19.09</td>
<td>0.293</td>
<td>8.19</td>
<td>8.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>18.76</td>
<td>0.295</td>
<td>8.30</td>
<td>9.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>18.60</td>
<td>0.295</td>
<td>8.35</td>
<td>9.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96</td>
<td>18.43</td>
<td>0.296</td>
<td>8.41</td>
<td>9.18</td>
</tr>
<tr>
<td>500 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>16.91</td>
<td>1.271</td>
<td>8.01</td>
<td>9.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>18.95</td>
<td>1.223</td>
<td>8.01</td>
<td>8.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>18.64</td>
<td>1.226</td>
<td>8.07</td>
<td>9.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>18.48</td>
<td>1.227</td>
<td>8.10</td>
<td>9.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96</td>
<td>18.33</td>
<td>1.229</td>
<td>8.14</td>
<td>9.12</td>
</tr>
<tr>
<td>3,000 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>16.84</td>
<td>5.453</td>
<td>7.92</td>
<td>9.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>18.89</td>
<td>5.556</td>
<td>8.01</td>
<td>8.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>18.53</td>
<td>5.581</td>
<td>8.06</td>
<td>8.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>18.34</td>
<td>5.593</td>
<td>8.08</td>
<td>8.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96</td>
<td>18.16</td>
<td>5.606</td>
<td>8.11</td>
<td>9.01</td>
</tr>
<tr>
<td>10,000 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>16.74</td>
<td>16.317</td>
<td>7.86</td>
<td>9.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>18.91</td>
<td>16.497</td>
<td>8.00</td>
<td>8.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>18.53</td>
<td>16.588</td>
<td>8.03</td>
<td>8.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>18.33</td>
<td>16.634</td>
<td>8.04</td>
<td>8.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96</td>
<td>18.14</td>
<td>16.680</td>
<td>8.06</td>
<td>8.65</td>
</tr>
<tr>
<td>30,000 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>16.59</td>
<td>44.497</td>
<td>7.67</td>
<td>8.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>19.00</td>
<td>45.007</td>
<td>7.94</td>
<td>7.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>18.69</td>
<td>45.140</td>
<td>7.92</td>
<td>7.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>18.53</td>
<td>45.207</td>
<td>7.91</td>
<td>7.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96</td>
<td>18.38</td>
<td>45.273</td>
<td>7.90</td>
<td>7.65</td>
</tr>
<tr>
<td>Water softener salt</td>
<td>13 November</td>
<td>18 November</td>
<td>Holding Tank</td>
<td>0</td>
<td>14.24</td>
<td>0.324</td>
<td>8.68</td>
<td>10.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>14.47</td>
<td>0.321</td>
<td>8.51</td>
<td>10.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>14.58</td>
<td>0.323</td>
<td>8.53</td>
<td>10.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>15.38</td>
<td>0.322</td>
<td>8.45</td>
<td>10.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96</td>
<td>15.14</td>
<td>0.317</td>
<td>8.32</td>
<td>10.05</td>
</tr>
<tr>
<td>0 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>10.79</td>
<td>0.314</td>
<td>8.26</td>
<td>10.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>17.75</td>
<td>0.313</td>
<td>8.18</td>
<td>9.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>17.81</td>
<td>0.312</td>
<td>8.16</td>
<td>9.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>17.97</td>
<td>0.313</td>
<td>8.15</td>
<td>9.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96</td>
<td>18.05</td>
<td>0.310</td>
<td>8.13</td>
<td>9.14</td>
</tr>
<tr>
<td>500 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>10.65</td>
<td>2.130</td>
<td>8.14</td>
<td>10.56</td>
</tr>
</tbody>
</table>
The estimated LD$_{50}$ value varied greatly depending on the exposure period (Table 3.2). For an exposure of 4 h, it would require a concentration of over 43,000 mg/l to kill half of the mussels, whereas, the concentration needed to kill half the mussels at a 24-h exposure was 11,405 mg/l. There was also a large range in the concentration required to cause 99% mortality. The concentration range for the LD$_{99}$ was 70,082 mg/l at 4 h to 15,646 mg/l at 24 h.
Figure 3.1. Average mortality (%) of adult zebra mussels (N=3 groups with 10 mussels in each group) from Otsego Lake after exposure to sodium chloride (NaCl) of varying concentrations in Fall 2014.

Table 3.2. Estimated LD$_{50}$ and LD$_{99}$ for adult zebra mussels exposed to sodium chloride with 95% confidence intervals for exposure durations of 4, 6, 12, and 24 hours and the sample dose(s) that caused 100% mortality during the experiment.

<table>
<thead>
<tr>
<th>Exposure Time (h)</th>
<th>LD$_{50}$ (95% CI) mg/L</th>
<th>LD$_{99}$ (95% CI) mg/L</th>
<th>SD$_{100}$ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>43607 (36552-52023)</td>
<td>70082 (58744-83608)</td>
<td>none</td>
</tr>
<tr>
<td>6</td>
<td>40028 (35057-45705)</td>
<td>62585 (54812-71461)</td>
<td>none</td>
</tr>
<tr>
<td>12</td>
<td>50512 (31022-344269)</td>
<td>552045 (142657-381190827)</td>
<td>none</td>
</tr>
<tr>
<td>24</td>
<td>11405 (10607-12263)</td>
<td>15646 (14551-16823)</td>
<td>30,000</td>
</tr>
</tbody>
</table>

Note: SD$_{100}$ indicates the sample dose (concentration) that caused 100% mortality for a given exposure time.
**Iodized Table Salt**

The mean length of mussels in this study group was 14.30 mm with an SD of 2.95 mm. The analysis of variance indicated a difference in mean lengths of the groups. The control group and the 10,000 mg/l concentration were found to be different from each other and the remaining concentrations. The concentrations of 500, 3,000, and 30,000 mg/l were found to have similar mean lengths. The mean length was not different between dead and live mussels ($F = 3.31$, $P = 0.0685$). The average shell lengths of the dead and live mussels were 14.61 and 14.24 mm, respectively.

Similar to the laboratory-grade sodium chloride, higher concentrations of table salt resulted in higher mortality than lower concentrations. The fastest exposure period that resulted in complete mortality was a 24-h exposure at 30,000 mg/l (Figure 3.2). Exposure periods longer than 24 h at that same concentration also caused complete mortality. The other concentration that caused 100% mortality was 10,000 mg/l at 72- and 96-h exposures. More than 50% mortality was caused by the 48-h exposure at 10,000 mg/l. The highest mortality caused by any other treatment was 3,000 mg/l with a 96-h exposure, which caused 43% mortality. The control group had two total mortalities, one each at the 1- and 96-h exposures.

![Figure 3.2](image-url)

**Figure 3.2.** Average mortality (%) of adult zebra mussels ($N=3$ groups with 10 mussels in each group) from Otsego Lake after exposure to iodized table salt of varying concentrations in Fall 2014.
The LD$_{50}$ and LD$_{99}$ concentrations for table salt showed a wide range (Table 3.3). The concentration needed to cause 50% mortality at the 4-h exposure was 2.54282E10 mg/l and the 24-h LD$_{50}$ was 12,698 mg/l. The LD$_{99}$ for a 24 h exposure was 42,173 mg/l.

Table 3.3. Estimated LD$_{50}$ and LD$_{99}$ for adult zebra mussels exposed to iodized table salt with 95% confidence intervals for exposure durations of 4, 6, 12, and 24 hours and the sample dose(s) that caused 100% mortality during the experiment.

<table>
<thead>
<tr>
<th>Exposure Time (h)</th>
<th>LD$_{50}$ (95% CI) mg/L</th>
<th>LD$_{99}$ (95% CI) mg/L</th>
<th>SD$_{100}$ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2.54282E10$^a$</td>
<td>6.14127E16$^a$</td>
<td>none</td>
</tr>
<tr>
<td>6</td>
<td>126012$^a$</td>
<td>2686409$^a$</td>
<td>none</td>
</tr>
<tr>
<td>12</td>
<td>115168 (40612-19198530)</td>
<td>12180309 (702349-7.61489E13)</td>
<td>none</td>
</tr>
<tr>
<td>24</td>
<td>12698$^a$</td>
<td>42173$^a$</td>
<td>30,000</td>
</tr>
</tbody>
</table>

Note: $^a$ indicates that no 95% confidence interval was generated. SD$_{100}$ indicates the sample dose (concentration) that caused 100% mortality for a given exposure time.

Water Softener Salt

Adults in this test had a mean shell length of 15.38 mm with an SD of 2.86 mm. There was no difference in shell lengths by concentration ($P = 0.37$) or between dead and live ($P = 0.26$) mussels. The average shell length of dead mussels was 15.20 mm and the average shell length of live mussels was 15.42 mm.

The fastest exposure period to cause complete mortality was 48 h at 30,000 mg/l (Figure 3.3). Exposures longer than 48 h also caused complete mortality at the concentration of 30,000 mg/l. The 10,000 mg/l concentration also caused complete mortality at exposures of 72 and 96 h. Concentration and exposure periods that led to at least 50% mortality were 30,000 mg/l for 12 and 24 h and 10,000 mg/l for 24 and 48 h. The control groups had complete survival at all exposure periods.

The LD$_{50}$ and LD$_{99}$ values for water softener salt varied greatly as well (Table 3.4). The LD$_{50}$ for a 40-h exposure was 170,851 mg/l and the LD$_{50}$ for 48-h exposure was 6,963 mg/l. The LD$_{99}$ value for a 4 h exposure was 43,228,644 mg/l and for a 48-h exposure it was 24,836 mg/l. The exposure period of 48 h was added because it was the first exposure period that had complete mortality for any concentration.
Figure 3.3: Average mortality (%) of adult zebra mussels (N=3 groups with 10 mussels in each group) from Otsego Lake after exposure to water softener salt of varying concentrations in Fall 2014.

Table 3.4. Estimated LD$_{50}$ and LD$_{99}$ for adult zebra mussels exposed to water softener salt with 95% confidence intervals for exposure durations of 4, 6, 12, 24, and 48 hours and the sample dose(s) that caused 100% mortality during the experiment.

<table>
<thead>
<tr>
<th>Exposure Time (h)</th>
<th>LD$_{50}$ (95% CI) mg/L</th>
<th>LD$_{99}$ (95% CI) mg/L</th>
<th>SD$_{100}$ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>$170851^a$</td>
<td>$43228644^a$</td>
<td>none</td>
</tr>
<tr>
<td>6</td>
<td>$484351^a$</td>
<td>$42534723^a$</td>
<td>none</td>
</tr>
<tr>
<td>12</td>
<td>$29004^a$</td>
<td>$158103^a$</td>
<td>none</td>
</tr>
<tr>
<td>24</td>
<td>$10434$ (8254-12936)</td>
<td>$35484$ (24542-76468)</td>
<td>none</td>
</tr>
<tr>
<td>48</td>
<td>$6963$ (5520-8716)</td>
<td>$24836$ (17148-50470)</td>
<td>30,000</td>
</tr>
</tbody>
</table>

Note: $^a$ indicates that no 95% confidence interval was generated. SD$_{100}$ indicates the sample dose (concentration) that caused 100% mortality for a given exposure time.
Discussion

The results of this study suggest that sodium chloride–based chemicals could be used for adult zebra mussel decontamination. Both the laboratory-grade sodium chloride and the iodized table salt caused complete mortality at the 30,000 mg/l concentration at the 24-h exposure period (Figure 3.4). The 24-exposure would be useful for when the user could wait a full day before using their equipment or watercraft. This could also be a reasonable use for people, companies, or agencies with multiple boats so that they can have access to a boat everyday if they follow an every-other-day rotation of their boats to allow enough time for full mortality of mussels. The use of sodium chloride–based water softener salt resulted in a 97% mortality after 24 h, compared with complete mortality in the other two sodium chloride–based chemicals in this test. This may be due to the impurities in the water softener salt compared with the high-grade sodium chloride and the iodized table salt. These results also show sodium chloride, table salt, and water softener salt caused complete mortality faster than in another study conducted by Ellis and MacIssac (2009). Both sequential and simultaneous changes in salinity were tested over a 48-h exposure period to mimic ballast water exchange and zebra mussel adults survived both forms of salinity change in the study (Ellis & MacIssac 2009). The maximum salinity obtained during both tests was 30,000 mg/l. In sequential salinity exchange, mussels were placed in the 30,000 mg/l concentration solution from the start and left in it for the length of the study period. In simultaneous salinity exchange, the salinity of the water was slowly increased from 4,000 mg/l to 30,000 mg/l over 4 h. The sequential salinity exchange is similar to the methods used in this study, so the results can be generally compared. The lengths of mussels used by Ellis and MacIssac are not available, so direct comparisons of the sample populations cannot be made relevantly. Another possible source of variation between studies is that saltwater was created using Instant Ocean, a salt used for creating salt water in aquariums (Ellis & MacIssac 2009). This synthetically made product may contain other ionic compounds in addition to sodium chloride, leading to a lower amount of sodium chloride present in the water for the same salinity measurement based on ionic measurement. Zebra mussels have been found to have a wide salinity tolerance that is unique to the water body they are in (McMahon 1996). So it may be possible to use the 10,000 mg/l concentration when the disinfecting watercraft after exposure to bodies of water that have mussels with low levels of salt intrusion, but it may not work in water bodies with higher salinities because the mussels may have acclimated to more saline conditions. It is also worth noting the solubility of sodium chloride in water compared with the values calculated for the LD50 and LD99 values. At 20°C, 35.6 g of sodium chloride cause 100 ml of water to become saturated. That is equivalent to 356,000 mg/l.
Figure 3.4: Average mortality (%) of adult zebra mussels (N=3 groups with 10 mussels in each group) from Otsego Lake after exposure to sodium chloride solutions at 30,000 mg/L in Fall 2014.

Iodized table salt was found to cause mortality at levels similar to the laboratory-grade sodium chloride. This can be beneficial from a cost standpoint. Laboratory-grade sodium chloride can cost around US$40/kg. To create 20 l of chemical solution, as was used in this study, it would cost more than $20. Iodized table salt for the study was purchased for US $0.86 for 737 g. This is a rate of US $1.17/kg. The savings caused by using iodized table salt would allow someone to create almost 18 times more of the solution compared with laboratory grade sodium chloride for the same cost. The size of the container that iodized table salt was sold in also allows for easy conversion for the lay person. One 737-g container can be poured into 5 gallons of water to create a solution that is slightly more concentrated than the highest concentration used in this study. Water softener salt may be an alternative for boaters who do not plan on using their boat for a longer period. Water softener salt is typically even cheaper (US $0.28/kg) than iodized table salt. It is sold in much larger quantities, usually about 40 lbs or 18.14 kg. This size package would certainly last longer, but would require one to measure the salt out for decontamination every time. There are 273 g of table salt in one U.S. cup. Assuming that the density is not extremely different between the table salt and softener salt, it would require less than 2.25 cups of softener salt to create 20 l of solution.

The use of sodium chloride to disinfect watercraft exteriors would be difficult because it would require a pond or holding tank where watercraft would have to be left for 24 h to cause
complete mortality of adult mussels. Adult mussels are macroscopic and can be removed from a boat hull with a scraper, as long as they are in a location that can be physically reached. Sodium chloride–based solutions would be useful for the treatment of gear such as ropes, chains, and anchors that can be completely immersed in the chemical treatment. Also, areas in watercraft that can hold water for extended periods of time, such as livewells and anchor boxes, could be disinfected by these solutions. The high salinity of these solutions could be corrosive to sensitive areas of watercraft so they may not work in all situations. It may be possible to rinse the area with either tap or well water to remove any mineral buildup after the needed exposure period is complete.

It has been suggested that zebra mussel adults have seasonal differences in filtration rates (Diggins 2001, Costa et al. 2008). Because of this, it was found that mussels may vary by a factor of up to 22 times between summer and winter, in susceptibility to chemical treatments based on those changes in filtration (Costa et al. 2008). Therefore, it would be beneficial to repeat this study at least once, in June, to determine if the zebra mussels in Otsego Lake are more susceptible to given concentrations of sodium chloride than they had been in the previous fall. A seasonal difference in susceptibility would allow for the use of fewer chemicals during a portion of the year that would decrease the prices of disinfection treatments. The change in susceptibility over time could explain the lower mortality caused by the softener salt. A test that examined the results of all three chemicals at the same time was not possible due to limited laboratory space. A concurrent test of all three chemicals in the future would be beneficial to eliminate any possible variation in susceptibility due to the difference in sample.
CHAPTER 4: TOXICITY OF POTASSIUM CHLORIDE COMPARED TO SODIUM CHLORIDE FOR DECONTAMINATION OF ZEBRA MUSSELS

Abstract

The use of chemicals to disinfect watercraft and equipment after exposure to zebra mussels is one decontamination method that is suggested by numerous government agencies in the United States. The ideal decontamination chemical would be easy to handle, easy to obtain, have limited non-target effects, be inexpensive, and relatively friendly to the environment. Potassium chloride and sodium chloride are two chemicals that have been tested separately in previous studies. The toxicity of both chemicals to adult mussels and veliger larvae was examined and compared in the current study. While both chemicals were effective at causing mortality within the study periods, potassium chloride was more effective than sodium chloride. Adult mussels experienced 100% mortality four times faster in potassium chloride than in sodium chloride at 30,000 mg/L and eight times faster at 10,000 mg/L. Complete mortality was reached in 12h at 1,250 mg/L by veligers in potassium chloride compared to 18h at 10,000 mg/L in sodium chloride. To determine if potassium chloride is more advantageous, the cost and chemical availability needs to be considered in addition to the circumstances of what needs to be decontaminated.

Introduction

Controlling the ongoing spread of aquatic invasive species (AIS) has become a very high priority for numerous natural resource agencies throughout North America (Zook and Phillips 2015). One species of great concern is the zebra mussel, *Dreissena polymorpha* (Pallas 1776), a bivalve native to the Ponto-Caspian region. Its rapid spread throughout the northeastern United States after its discovery in the 1980s, combined with its ability to completely alter the ecosystem it invades, has caused many western US agencies to develop watercraft interception programs (Zook and Phillips 2015). These programs are designed to prevent the spread of aquatic invasive species that are on trailered watercraft, seaplanes, and other equipment in order to preserve natural resources (DiVittorio 2015, Zook and Phillips 2015). One major aspect of the watercraft interception programs is the actual inspection of those watercraft and the determination of what to do if the boat is determined to be a possible source of AIS spread. Trailered watercraft are believed to be one of the greatest vectors for the spread of AIS (Johnson et al. 2001). Once a vessel has been deemed a “threat” to the spread of zebra mussels, there are a few recommended courses of action. They include chemical treatment, heat, hot water/high pressure washing, freezing, physical removal, and desiccation (DiVittorio et al. 2012). When it comes to those treatment options, there are only a few that agencies can expect watercraft owners to perform themselves without needing any specialized equipment or training. Desiccation is the easiest and least expensive form of decontamination for aquatic organisms but can require greater lengths of time than boat owners are willing to wait (i.e. up to 40 days for attached adult
mussels) before reusing their boats (DiVittorio et al. 2012). Physical removal of attached mussels does not require any specialized equipment but is only really applicable to the life stages of mussels that are visible to the naked eye. It can be a very labor intense process to remove attached mussels that have colonized large areas or hard-to-reach locations (DiVittorio et al. 2012). Freezing is another easy decontamination method when ambient temperatures are low enough to cause freezing, otherwise one would need something like dry ice crystals to spray over the mussels to cause them to freeze. Also, when multiple layers of mussels are present, greater lengths of time may be required to freeze the innermost layer of mussels (McMahon et al. 1993). Chemical treatments can be recommended to watercraft owners but have to come with some caveats. One drawback to chemical treatments is what to do with the chemical after the treatments. They often require a disposal plan (DiVittorio et al. 2012). Another potential problem is that some chemicals that have been shown to be toxic to adult mussels or veligers are sometimes registered pesticides that require special storage or handling. Other chemicals can be effective but their use results in harmful byproducts (Watters et al. 2013). However there are some chemicals that have been recommended for use that can be easily obtained by the general public, are relatively inexpensive, and are safe to handle. Two chemicals that fit this description are potassium chloride and sodium chloride.

Other studies have tested either potassium chloride and/or sodium chloride as chemical treatments on either adult mussels, veliger larvae, or both (Davis et al. 2015a, Edwards et al. 2000, 2002, Fernald and Watson 2013, Fisher et al. 1994, Lewis et al. 1997, Pucherelli et al. 2014, Sykes 2009, Waller et al. 1996, Wildridge et al 1998). Fisher et al. (1991) found that potassium was toxic to zebra mussels at elevated concentrations. They reported vacuolization of the epithelial cells of the gills of mussels exposed to elevated potassium levels and suggested the pathology was likely related to loss of fluid and/or electrolyte balance in the cells due to functional or structural changes in the plasma membrane (Fisher et al. 1991). However, the direct comparison of the toxicity of potassium chloride and sodium chloride to adult zebra mussels and veliger larvae has not been reported previously. The current study set out to compare the toxicity of those two chemicals with findings to each other and the previous findings. It was hypothesized that both chemicals would cause mortality to adult mussels and veliger larvae but potassium chloride would cause mortality sooner at the same concentration and at lower concentrations for similar exposure periods.

Methods

Adult Collection and Acclimation

Rocks colonized by adult mussels were collected at the State University of New York College at Oneonta Biological Field Station (BFS) Thayer Boathouse on Otsego Lake, New York, in water around 1m in depth using a variety of clam rakes. The rocks were brought back to
the lab in trays and coolers. Mussels were removed from the rocks using a paint scraper (similar to Costa et al. 2008) and were placed into a small tray with a constant flow of fresh Otsego Lake water. Mussels bunched together via byssal threads were pulled apart and placed into the tray. Once all the mussels were in the tray, eleven mussels were selected at random and placed into an 800-micron mesh bag. Those with any physical damage were discarded. One hundred and fifty bags were filled with mussels. All extra individuals were placed into a separate bag. The bags were placed into a large aquarium (~150 L) with a slow constant flow of lake water for at least 72 hours for the mussels acclimate to the bags and to determine any mortality due to handling and/or stress (similar to Comeau et al. 2011). The aquarium was lightly aerated with compressed air. After the 72-hour holding period, the mesh bags were removed one at a time and the mussels inside were examined for mortality. Any dead individuals were removed from the bags. If no mortality occurred, one mussel was selected at random, removed from the bag, and discarded. All bags had 10 included individuals so that there were 30 individuals for every concentration-exposure period combination. The number of mortalities for all treatments during the acclimation period was less than 1%.

Chemical Treatment

Fifteen glass tanks were rinsed with lake water and scrubbed with an abrasive sponge soaked in lake water. The tanks were then emptied, rinsed, and emptied again. Twenty liters of Otsego Lake water was added to each tank. The required amount of each chemical was measured on an electronic scale and then added to each tank. The chemicals that were tested were produced by Amresco (Solon, OH). Four chemical concentrations were used for the treatments; they were 500, 3,000, 10,000, and 30,000 mg/L. The highest concentration of sodium chloride (30,000 mg/L) was chosen to represent the equivalent of seawater. Also, 10,000 mg/L of sodium chloride was the concentration found to be effective on zebra mussel veligers and settlers (mussels less than 2 mm in length) by Waller et al. (1996). Once the salt was added to the water in each tank, the water was stirred with a wooden dowel until the chemical was completely dissolved. There were three duplicates of each concentration as well as three control tanks with no chemicals. Air stones were added to each tank and were lightly aerated with compressed air to ensure continued mixing of the treatment water. Once all tanks had been filled and mixed, ten bags of mussels were hung from a wooden dowel in each aquarium. Each bag represented an exposure time period (0, 1, 2, 4, 6, 12, 24, 48, 72, and 96 h). After the bags in all tanks were immersed, the bags of mussels that represented 0 h were then removed from each tank. Once bags were removed from the treatment tanks, they were labeled with their exposure time and hung from wooden dowels in two large holding tanks with continuously flowing water from Otsego Lake. This created an environment that was very close to the natural conditions. One dowel held all the bags from an individual tank. Mussels were left in this tank for at least 48 h for recovery. Mussels were examined for mortality between 48 and 72 h after being removed from their chemical treatments. This was done because it has been shown that mussels that
appear dead at the end of a chemical treatment can recover after being placed in clean water free of chemicals (Pucherelli et al. 2014, Wildridge et al. 1998). Mortality was determined by the mussel exhibiting gaping valves when removed from the mesh bag and placed on a paper towel. Mussels with a slight gape had a blunt probe placed between the valves and if the mussel closed it was considered alive. A mussel with no movement of the valves after probing was considered dead. Only mussels with a slight valve gape were probed, or when more than half of a sample group was fully gaping showing mortality. After mortality assessment, the shell length of each mussel was measured using a digital caliper (Model Number: CD-6”CX, Mitutoyo Corporation, Kawasaki, Japan) and recorded along with the mortality status.

Water Quality
An YSI (Model Number: 6820V2-M, YSI Incorporated, Yellow Springs, Ohio) multiparameter water quality measurement was used in each chemical treatment tank and the recovery/holding tank at times 0, 24, 48, 72, and 96 h. Prior to each measurement, the YSI was calibrated following standard operation protocols to ensure probe accuracy. All tanks of the same concentration were measured starting from the control tanks and increasing in concentration. The probe was rinsed with lake water between concentration levels. The parameters measured were temperature, specific conductivity, pH, and dissolved oxygen. Measurements were taken from the chemical treatment tanks after mussels had been removed for the same exposure time. Measurements in the recovery tank were taken after bags of mussels were added to the tank. This was to measure the maximum amount of chemical being added to the holding tank via chemical solution soaked into the mesh bags or left on/in adult mussels. The constant flow of fresh lake water in the recovery tank would replace the water in the tank over time diluting the chemical.

Veliger Collection
Zebra mussel veligers were collected from the top 3 m of the water column of Otsego Lake, NY using horizontal tows (about 750 m per tow) with a 63-µm plankton-net. The contents of the net were placed into an opaque 1L bottle. Multiple tows were performed until the bottle was at least 75% full, then they were taken to the main laboratory at the Biological Field Station. Once back at the lab, some of the contents of the bottle were placed into a 500-mL beaker and were concentrated using a cup with a bottom of 63 µm net material. This was repeated until all of the contents of the bottle had been concentrated to about 100 mL of liquid. A gridded Sedgewick-rafter cell was used to enumerate the veligers in 1 mL of concentrated sample water using cross-polarized light (CPL) microscopy. This was repeated two more times and the average number of veligers per milliliter was calculated. A concentration of 50-100 veligers per milliliter of sample was desired so the sample would be further concentrated or diluted with filtered lake water until this goal was met. A sample of veligers collected was preserved in ethanol for analysis of the distribution of veliger stages and sizes.
**Veliger Chemical Treatment**

In a 50 mL beaker, 24 mL of chemical solution was made by combining lake water filtered through 63 µm net material with the corresponding amount of chemical needed to make 25 mL of solution for each concentration level (0, 1,250, 2,500, 5,000 mg/L for potassium chloride and 0, 5,000, 10,000, 15,000 mg/L for sodium chloride) that was tested. There were three beakers for every concentration and exposure time (18 and 24 h for sodium chloride and 12, 18, and 24 h for potassium chloride) combination during each test. After all of the beakers had chemical solution within them, 1 mL of concentrated sample water was added to each beaker using a pipette with the tip cut to prevent damage to the veligers. At the end of the exposure time, each beaker was poured into a veliger holding device (VHD) and then rinsed with filtered lake water and poured into the device again. Each VHD was labelled with the chemical name, concentration, and exposure time and was placed into a recovery tank of filtered lake water that was lightly aerated with compressed air. The VHDs were constructed by drilling a one-quarter inch hole in the cap of a 15 mL centrifuge tube, removing the screw-on cap and placing 63-µm net material over the end of the tube, putting the cap back on, and finally cutting the pointed end of the centrifuge tube off. This design allowed the veligers to be constrained to a small area so that it would be easy to recollect them to observe for mortality while ensuring that they were exposed to aerated chemical-free water. Some chemicals have been shown to cause veligers to appear dead immediately following chemical exposure but after being placed into water without the chemical, the veligers can recover (Pucherelli et al. 2014). Veligers were observed for mortality following removal from the chemical solution and placement into the recovery tank using the Sedgewick-rafter cell and CPL microscopy. One milliliter was observed from each veliger holding device and the number of dead and live veligers was counted. All samples for each concentration-exposure time were observed consecutively (i.e. replicate 1 then replicate 2 then replicate 3 for 0 mg/L-18h NaCl). The total number of all veligers for the three replicates was determined and if the total did not exceed 30 veligers, another milliliter was observed from each replicate. In samples with more than 100 veligers, only the first 100 veligers observed were used for mortality assessment. After this observation, the contents of the slide were rinsed back into the VHD with filtered lake water. This was repeated every 24 h until the control groups reached greater than 50% mortality or 72 h, whichever occurred first. Veligers were considered to be dead if they met any of the following criteria: no internal organs visible within the shell, internal organs leaking out of the shell, no movement of internal organs, and no movement of cilia (*sensu* Watters et al. 2013).

**Statistical Analysis**

All data were entered into a Microsoft Excel spreadsheet. Descriptive statistics were run on the shell length of all mussels. Excel was also used to determine the average mortality for each concentration at each time interval and to create a graph showing mortality at each interval. Adult mortality data was used to create the LD$_{50}$ and LD$_{99}$ with 95% confidence intervals for
times 4, 6, 12, and 24 h for each chemical using a probit regression. The \( \text{LD}_{50} \) was the concentration of chemical that would result in 50% mortality of mussels for the selected exposure time and the \( \text{LD}_{99} \) was the concentration of chemical that would result in 99% mortality of mussels for the selected exposure time. It was determined that 24 hours would be the longest time that would be considered a reasonable amount of time for a vessel to be decontaminated with chemical treatments. To compare the effectiveness of the chemicals, an analysis of covariance (ANCOVA) was performed with the concentration as the covariate for each exposure interval (Zar 1996). For each chemical treatment, an analysis of variance (ANOVA) was also used to compare shell length of adult mussels between mussels that were killed and those that survived. A t-test was performed to compare the mean shell length of adult mussels from the potassium chloride test and the sodium chloride test. The average observed mortality (%) from the veliger mortality assessments was calculated for each concentration and exposure time combination. The mortality data were transformed with an arc-sine transformation due to non-normal distribution and low replicate number. To compare the effectiveness of the two treatments, an ANCOVA was performed using the 5,000 mg/L concentration mortality, as it was the only concentration used for both tests, with exposure time as the covariate. The level of significance was set at \( \alpha = 0.05 \) for all tests. Statistical analyses were performed with SAS® (Version 9.3 SAS Institute Inc., Cary, NC).

**Results**

*Potassium chloride-adult mussels*

The mean length of mussels in the study was 13.57 mm with a standard deviation of 3.70 mm. The average shell length of the dead mussels was not significantly different than the average length of the live mussels, nor did shell length vary by chemical concentration (ANCOVA, \( F = 0.51, P=0.77 \)).

As expected, higher concentrations of KCl caused mortality at a faster rate than lower concentrations (Figure 4.1). Exposures of 4 or fewer hours did not cause 100% mortality at any concentration. The quickest exposure time to create complete mortality was 6 hours at 30,000 mg/L. It also caused 100% mortality at 12 hours. The next concentration-exposure time to cause 100% mortality was 10,000 mg/L at 12 hours. Both 30,000 mg/L and 10,000 mg/L caused complete mortality at 24 hours of exposure. No other concentration caused complete mortality in less than 48 hours. The control samples had 100% survival. All test concentrations did show some degree of mortality at exposures below 24 hours.

The estimated \( \text{LD}_{50} \) value varied greatly depending on the exposure interval (Table 4.1). For an exposure of 4 hours, it would require a concentration of almost 13,000 mg/L to kill half of the mussels. Whereas the concentration needed to kill half the mussels at a 24 hour exposure is
under 278.9 mg/L. There was also a large range in the concentration required to cause 99% mortality. The concentration range for the LD₉₀ was 45,865 mg/L at 4 hours to 25,084 mg/L at 24 hours. The water quality parameters for the treatment tanks were all very similar for the duration of the experiment.

Figure 4.1. Mortality (%) of adult zebra mussels (N=3 groups, each with 10 mussels) from Otsego Lake, NY after exposure to potassium chloride (KCl) of varying concentrations in Fall 2014.

Table 4.1. Estimated LD₅₀ and LD₉₀ for adult zebra mussels from Otsego Lake, NY exposed to potassium chloride with 95% confidence intervals for exposure durations of 4, 6, 12, and 24 hours and the sample dose(s) that caused 100% mortality during the experiment.

<table>
<thead>
<tr>
<th>Exposure Time (h)</th>
<th>LD₅₀ (95% CI) mg/L</th>
<th>LD₉₀ (95% CI) mg/L</th>
<th>SD₁₀₀ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>12916 (10285-16091)</td>
<td>45865 (31429-98153)</td>
<td>none</td>
</tr>
<tr>
<td>6</td>
<td>7217 (5410-9549)</td>
<td>50750 (29859-137900)</td>
<td>30,000</td>
</tr>
<tr>
<td>12</td>
<td>1067 (737.3-1492)</td>
<td>8213 (4703-23130)</td>
<td>10,000, 30,000</td>
</tr>
<tr>
<td>24</td>
<td>278.9 (40.65-610.2)</td>
<td>25084 (8656-463332)</td>
<td>10,000, 30,000</td>
</tr>
</tbody>
</table>

SD₁₀₀ indicates the sample dose that caused 100% mortality for a given exposure time.
Sodium chloride-adult mussels

The mean length of mussels in this study group was 13.98 mm with a standard deviation of 2.96 mm. The mean length of the dead mussels was significantly different than the mean length of the live mussels exposed to different concentrations of sodium chloride (ANCOVA, F = 12.15, P = <0.0001). The mean length of the control group was 15.03 mm compared to the other four concentrations (range of 13.60-13.89 mm). The control group was removed and the mussels from the treatments were used to compare the mean lengths of the live and dead mussels. No difference was found with the control group removed (ANCOVA, F =3.35, P = 0.0674). The average shell length of dead mussels was 13.41 mm and the average shell length of the live mussels was 13.79 mm.

No exposure time less than 24 hours caused complete mortality (Figure 4.2). Higher concentrations of sodium chloride resulted in higher mortality than lower concentrations for the exposure periods longer than 6 hours. The fastest exposure period that resulted in complete mortality was a 24 hour exposure at 30,000 mg/L. Exposure periods longer than 24 hours at the same concentration caused complete mortality. The only other concentration that caused 100% mortality was 10,000 mg/L with a 96 hour exposure. The other treatments that caused more than 50% mortality were the 72 hour and 48 hour exposures at 10,000 mg/L. The highest mortality caused by any other treatment was 3,000 mg/L with a 96 hour exposure, which caused 20% mortality.

The estimated LD$_{50}$ value varied greatly depending on the exposure period (Table 4.2). For an exposure of 4 hours, it would require a concentration of over 43,000 mg/L to kill half of the mussels, whereas the concentration needed to kill half the mussels at a 24 hour exposure was 11,405 mg/L. There was also a large range in the concentration required to cause 99% mortality. The concentration range for the LD$_{99}$ was 70,082 mg/L at 4 hours to 15,646 mg/L at 24 hours.

Chemical comparison-adult mussels

The mean shell length of the mussels in each test were not similar (N = 3,000, t-test, t = 3.30, p-value <0.001). The mean lengths were different by 0.4 mm. The testing chemical was a significant factor for mortality for every exposure period other than 0 h (Table 4.3). The concentration of chemical was also a significant factor at every exposure period other than 0 h. The interaction of the chemical and the concentration was a significant factor for exposure periods other than 0, 12, and 24 h.

Potassium chloride-veliger

Veligers in the experimental groups appeared to have complete mortality for the first 48 hours following removal from the chemical treatments (Table 4.4). During the 72 h post-removal mortality assessment, the veligers exposed to 500 mg/L potassium chloride had some recovery.
The average mortality rate was 98.48, 99.19, and 98.55% for the 12, 18, and 24 h exposure periods, respectively. The control groups had below 40% mortality for the mortality assessments for 48 h following removal and had average mortality greater than 50% at the 72 h mortality assessment.

![Figure 4.2](image.png)

Figure 4.2. Average mortality (%) of adult zebra mussels (N=3 groups, each with 10 mussels) from Otsego Lake, NY after exposure to sodium chloride (NaCl) of varying concentrations in Fall 2014.

Table 4.2. Estimated LD$_{50}$ and LD$_{99}$ for adult zebra mussels from Otsego Lake, NY exposed to sodium chloride with 95% confidence intervals for exposure durations of 4, 6, 12, and 24 hours and the sample dose(s) that caused 100% mortality during the experiment.

<table>
<thead>
<tr>
<th>Exposure Time (h)</th>
<th>LD$_{50}$ (95% CI) mg/L</th>
<th>LD$_{99}$ (95% CI) mg/L</th>
<th>SD$_{100}$ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>43607 (36552-52023)</td>
<td>70082 (58744-83608)</td>
<td>none</td>
</tr>
<tr>
<td>6</td>
<td>40028 (35057-45705)</td>
<td>62585 (54812-71461)</td>
<td>none</td>
</tr>
<tr>
<td>12</td>
<td>50512 (31022-344269)</td>
<td>552045 (142657-381190827)</td>
<td>none</td>
</tr>
<tr>
<td>24</td>
<td>11405 (10607-12263)</td>
<td>15646 (14551-16823)</td>
<td>30,000</td>
</tr>
</tbody>
</table>

SD$_{100}$ indicates the sample dose that caused 100% mortality for a given exposure time.
Table 4.3. ANCOVA results of lethal effects of KCl and NaCl treatments on adult mussels.

<table>
<thead>
<tr>
<th>Exposure Period (h)</th>
<th>Source</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Concentration</td>
<td>0.51</td>
<td>0.4796</td>
</tr>
<tr>
<td></td>
<td>Chemical</td>
<td>0.97</td>
<td>0.3349</td>
</tr>
<tr>
<td></td>
<td>Chemical x concentration</td>
<td>0.51</td>
<td>0.4796</td>
</tr>
<tr>
<td>1</td>
<td>Concentration</td>
<td>114.39</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Chemical</td>
<td>31.49</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Chemical x concentration</td>
<td>96.21</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>2</td>
<td>Concentration</td>
<td>142.72</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Chemical</td>
<td>39.34</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Chemical x concentration</td>
<td>123.14</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>4</td>
<td>Concentration</td>
<td>297.79</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Chemical</td>
<td>141.12</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Chemical x concentration</td>
<td>309.14</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>6</td>
<td>Concentration</td>
<td>70.20</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Chemical</td>
<td>1.19</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Chemical x concentration</td>
<td>53.44</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>12</td>
<td>Concentration</td>
<td>19.24</td>
<td>0.0002*</td>
</tr>
<tr>
<td></td>
<td>Chemical</td>
<td>12.50</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Chemical x concentration</td>
<td>3.46</td>
<td>0.0742</td>
</tr>
<tr>
<td></td>
<td>Concentration</td>
<td>50.38</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>24</td>
<td>Chemical</td>
<td>28.41</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Chemical x concentration</td>
<td>3.36</td>
<td>0.0783</td>
</tr>
</tbody>
</table>
Table 4.4. Average observed mortality of zebra mussel veligers from Otsego Lake, NY exposed to potassium chloride of varying concentrations at periods following removal from treatments.

<table>
<thead>
<tr>
<th>Exposure (h)</th>
<th>Concentration (mg/L)</th>
<th>Average observed mortality (%) of veligers at times after removal from treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>38.15%</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>1,250</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>2,500</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>100.00%</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>16.61%</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>1,250</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>2,500</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>100.00%</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>16.80%</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>1,250</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>2,500</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Sodium chloride-veliger

The veligers in the control group had an average mortality below 35% for the 0 and 24 h post-removal mortality assessments for both exposure periods (Table 4.5). The 18 h exposure control group had an average mortality of 37.84% at the 48 h post-removal assessment, while the 24 h control group had an average mortality of 85.45% at the same assessment. Veligers in the 5,000 mg/L concentration had some recovery at the 48 h post-removal mortality assessment with average observed mortalities of 97.84% for the 18 h exposure and 99.56% at the 24 h exposure. The 10,000 and 15,000 mg/L concentrations had complete mortality for both exposure periods at every post-removal mortality assessment with no veliger recovery.

Chemical comparison-veliger

The ANCOVA indicated that chemical treatment and exposure time were significant factors for veliger mortality (F = 30.28, p-value < 0.0001) and potassium chloride was more lethal than sodium chloride (p-value = 0.0476). The treatment-exposure time interaction was not significant (p-value = 0.2340).
Table 4.5. Average observed mortality of zebra mussel veligers from Otsego Lake, NY exposed to sodium chloride of varying concentrations at periods following removal from treatments.

<table>
<thead>
<tr>
<th>Exposure (h)</th>
<th>Concentration (mg/L)</th>
<th>Average observed mortality (%) of veligers at times after removal from treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>22.47%</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>98.67%</td>
</tr>
<tr>
<td></td>
<td>10,000</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>15,000</td>
<td>100.00%</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>23.05%</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>10,000</td>
<td>100.00%</td>
</tr>
<tr>
<td></td>
<td>15,000</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

The majority (80%) of veligers during the tests were D-stage (Figure 4.3). Umbonal stage veligers were 16% of the sample, while pediveligers comprised only 4%.

Figure 4.3. Length-frequency distribution of zebra mussel veligers (N=51) removed from Otsego Lake, NY when testing was performed.
Discussion

Potassium chloride caused complete mortality of adult mussels at every concentration in under 24 h. A 6 hour exposure to 30,000 mg/L was found to cause complete mortality, with the estimated LD₉₀ at a little over 50,000 mg/L. The confidence interval was as low as 29,859 mg/L. This would be useful for people who want to be able use their watercraft or equipment in a different water body than the one they originally used. The 30,000 mg/L treatment also caused complete mortality at the 12 hour exposure timeframe, as did the 10,000 mg/L treatment. The LD₅₀ was 278.9 mg/L (40.65-610.2 95% confidence interval) for the 24h exposure in the current study. This was slightly higher than 138 mg/L (123-161) found by Fisher et al. (1991). However, the two results are not significantly different as the 95% confidence intervals overlap. Another study using potassium chloride on adult mussels examined using 100 mg/L at a variety of temperatures to determine the time required to cause 95% mortality based on the temperature of the water used. For room temperature (~20°C), it was estimated to take 56 h to cause 95% mortality and 165 h at 12-14°C (Lewis et al. 1997). Areas that are sensitive to corrosion due to their material construction can be treated with a lower concentration for a longer exposure time. Decreased concentrations with longer exposure periods may also be useful for electrical components that may have problems when exposed to solutions with a high ionic concentration. It was found that a 24 hour exposure to 10,000 mg/l was completely lethal to adult mussels with the estimated LD₉₀ just over 25,000 mg/l with the confidence interval including concentrations as low as 8656 mg/L. This still allows for complete mortality of adult mussels within a reasonable timeframe to return to using the watercraft or equipment. This would also allow for a decreased cost of decontamination.

In our previous study on three forms of sodium chloride (Davis et al. 2015a) it is suggested that sodium chloride could be used for adult zebra mussel decontamination. Zebra mussels have been found to have a wide salinity tolerance that is unique to the water body they are in (McMahon 1996). It may be necessary to conduct toxicity testing with sodium chloride using mussels collected from bodies of water representing diverse saline levels to provide more accurate recommendations for decontamination protocols.

When comparing the effectiveness of both chemicals for adult mussel decontamination, potassium chloride was more effective than sodium chloride for every exposure period other than 0 h, as determined by the ANCOVA. For the highest concentration tested, potassium chloride caused complete mortality in one-fourth of the time of the sodium chloride. Concentration was also a significant cause of mortality, which can be expected because lower concentrations typically will cause less mortality than higher concentrations for the same exposure period no matter what chemical is being used. The size difference between the mussels of the two experiments was minimal (0.4 mm) but significant. The large sample size likely caused the significant difference in size (N = 3,000). However, all mussels that were tested were adult
mussels (<8 mm) and were collected from the same location within a few weeks of each other. Therefore, influence of mussel size on testing results is likely minimal.

Potassium chloride caused complete mortality without any veliger recovery at levels that have been reported by some others to be effective. A concentration of 2,500 mg/L caused complete mortality on zebra mussel veligers at 24 h taken from Lake Erie (Waller et al. 1996). Pucherelli et al. (2014) found an exposure of 2,500 mg/L of potassium chloride for 24 h to be effective on quagga mussel veligers with and without a formalin secondary treatment. They also found 50 mg/L of potassium chloride with a formalin secondary treatment to be effective on quagga mussel veligers (Pucherelli et al. 2014). In another study, a 750 mg/L for 1 h treatment plus a formalin secondary treatment was reported to be effective against zebra mussel veligers, but that study did not include a recovery period (Edwards et al. 2000, 2002). The exclusion of a recovery period in that study raises concerns over the possible recovery of veligers after being placed into chemical-free water, similar to what was observed in the current study. However, some other studies have found potassium chloride to be ineffective at levels similar to those tested in the current study. Sykes (2009) found 4,250 mg/L of potassium chloride with a formalin secondary treatment was not effective at causing mortality of quagga mussel veligers. The lack of effectiveness was suggested to be due to the elevated hardness of the water from where the veligers were collected, and also due to the water used during the chemical treatments (Sykes 2009).

Sodium chloride was found to be an effective chemical for the treatment of veligers. The exposure periods required for complete mortality with sodium chloride do not allow for a rapid decontamination but would be sufficient for many other situations. The results were consistent with previously reported results. A concentration of 10,000 mg/L was found to cause 100% mortality at an exposure period of 24 h when the water temperature was 12°C (Waller et al. 1996). The current study found 10,000 mg/L of sodium chloride salt were effective at both the 18 h and 24 h exposures. The water temperature in the current study was closer to 20°C. The higher water temperature may have contributed to a higher mortality rate by causing an increase in veliger metabolism that caused a greater rate of chemical ingestion by the veligers. The 10,000 mg/L concentration was found to be safe for multiple species of fish at the 24 h exposure at both 12°C and 17°C (Waller et al. 1996). The goal of that study was to determine a safe decontamination method to be used during aquaculture practices. During that same study, a 20,000 mg/L concentration of sodium chloride was found to cause complete mortality to zebra mussel veligers with a 6 h exposure period with a 17°C temperature (Waller et al. 1996). In a study looking at chemical control of quagga mussel veligers, the 10,000 mg/L concentration of sodium chloride was not effective at the 24 h exposure (Pucherelli et al. 2014). This difference in results may be due to interspecific differences in the veliger or in differences in the sources of
the samples. The water used by Pucherelli et al. (2014) had a much higher ionic concentration than the water from Otsego Lake.

Potassium chloride was more effective than sodium chloride for use as a veliger decontamination tool. The type of treatment was a significant indicator of mortality in the ANCOVA, as was exposure period. The exposure period was likely significant because potassium chloride caused complete mortality at a shorter exposure (12 h) while sodium chloride did not have complete mortality observed following veliger removal from the chemical treatments. It is not surprising that potassium chloride was more effective than sodium chloride because potassium was shown to cause damage to the epithelial cells of mussel gills by Fisher et al. (1991).

While potassium chloride is often more expensive than sodium chloride, the overall increase in effectiveness at decontamination compared to sodium chloride is likely a greater trade-off. The ability to have gear and equipment ready for use sooner could result in an increased likelihood of decontamination protocols being followed by recreational watercraft owners. This could also lead to an increase in productivity of those collecting scientific data. Potassium chloride is sometimes recommended because it is considered to be less corrosive than sodium chloride. This does not mean that potassium chloride is non-corrosive. A potassium chloride solution was created and used for decontamination gear and equipment during the summer of 2015 by the intern program at the SUNY Oneonta Biological Field Station. Items that were left in the solution for extended periods of time (i.e. days), surfaces that are susceptible to corrosion were often heavily corroded upon removal from the solution. Iodized table salt was shown to work as effectively as high grade sodium chloride, which would allow for decontamination costs to be decreased significantly when using sodium chloride (Davis et al. 2015a). A potassium chloride-based table salt alternative was found to cause mortality to adult mussels that was similar to that of the reagent grade potassium chloride used during the current study (Davis EA, unpublished data). This also can reduce decontamination costs. Potassium chloride based water softener salt could offer an even cheaper alternative for using a potassium chloride based chemical treatment, however testing should be conducted to determine if it is effective on zebra mussels.

Due to the low number of replicates that were performed during this study, another study that uses more replicates would allow for greater statistical analysis of the results and strengthen the ground from which decontamination protocols are decided. The results found in the current study should guide future work that is focused on preventing the spread of zebra mussels.
CHAPTER 5: DISTILLED WHITE VINEGAR (5% ACETIC ACID) AND VIRKON AQUATIC FOR RAPID ZEBRA MUSSEL VELIGER DECONTAMINATION

Abstract
The ongoing search for a chemical treatment that can be recommended for use to combat the spread of aquatic invasive species has led to the testing of a wide array of chemicals. Virkon Aquatic and distilled white vinegar, having been reported as effective on mussel veligers, were tested on zebra mussel veligers taken from Otsego Lake, NY. Distilled white vinegar was tested to verify the recommendations of DiVittorio et al. 2012. Virkon Aquatic was chosen to compare its toxicity to zebra mussel veligers with its toxicity to quagga mussel veligers. Virkon Aquatic was found to be effective at a concentration 0.5 % for an exposure of 2 min. Distilled white vinegar was effective at a concentration of 25% vinegar (1.25% acetic acid) with a 10 min exposure. The ability of these chemicals to be effective at relatively short exposure periods could lead to an increase in the prevention of the spread of zebra mussels. Treatment solutions created with either chemical have potential to be reused multiple times, allowing for decreased cost associated with using them for watercraft disinfection.

Introduction
After its discovery in the Great Lakes in the 1980s, the zebra mussel, Driessena polymorpha, (Pallas, 1776) quickly spread throughout the northeast and midwestern United States in waterways connected to the Great Lakes, such as the Mississippi River and the New York Canal System (Mackie and Claudi 2010). Much of their rapid expansion is attributed to their larval life stage, known as the veliger stage. Veligers are not readily seen by the naked eye and they are planktonic. Due to the difficulty of detection, a large number of veligers can be transported in a relatively small amount of water, such as the remnant water left in the bilge or livewell of a trailered watercraft. Choi et al. (2013) found that veligers of quagga mussels, Dreissena rostriformis bugensis (Andrusov, 1897), were able to survive up to 27 days during autumn and 3 days in the summer in the equivalent amount of water typically left in a trailered watercraft. Overland transport of veligers is generally accepted as the main cause of the rapid spread of the zebra mussel (Johnson et al. 2001). In response to the threat posed by trailered watercraft as vectors for zebra mussel introductions, many natural resource agencies began watercraft interception programs to stop “high risk” boats from being launched into mussel-free water bodies (Zook and Phillips 2015). An issue that arises when a “high risk” watercraft is identified is how to eliminate the risk of spreading zebra mussels (DiVittorio 2015). Extensive testing, using a wide variety of techniques, has been performed to attempt to completely decontaminate watercraft. Currently there are six methods of decontamination that are recommended by the United States Bureau of Reclamation. They are chemical treatment, heat, hot water/high pressure washing, freezing, physical removal, and desiccation (DiVittorio et al. 2012). Chemical treatment is a method that is generally focused on the veliger life stage of the
zebra mussel. Adult mussels can detect some chemicals, such as chlorine, and close their valves in response, allowing them to stay alive for longer periods of time by avoiding the chemical (Rajagopal et al. 1997, 2002). Adult mussels would therefore have to be submerged in the chemical treatment for extended periods of time so that they eventually have to open their valves to avoid hypoxia-induced mortality and are exposed to the chemical.

Two chemicals that have been recommended for decontamination of zebra mussel infested watercraft are distilled white vinegar (DiVittorio et al. 2012) and Virkon Aquatic (Moffitt et al. 2015). DiVittorio et al. (2012) recommended using undiluted distilled white vinegar for an exposure of 20 min to disinfect equipment exposed to mussel veligers. However, there was no data set provided to support how this conclusion was reached. Davis et al. (2015) found distilled white vinegar (5% acetic acid) to be effective at causing mortality in adult zebra mussels in relatively short periods depending on the concentration. This study continues that of Davis et al. (2015) by testing whether diluted concentrations of vinegar can cause mortality in zebra mussel veligers. Virkon Aquatic is a registered disinfectant that is used in aquaculture facilities (Dupont 2006a). It was tested on quagga mussel veligers and found to be effective at causing complete mortality at a concentration of 5 g/L, or 0.5%, in 5-10 min (Moffitt et al. 2015). The goal of the present study was to determine the toxicity of distilled white vinegar and Virkon Aquatic at varying concentrations to zebra mussel veligers.

**Methods**

**Veliger Collection**

Zebra mussel veligers were collected from the top 3 m of Otsego Lake, NY using horizontal tows (approximately 750 m per tow) with a 63 µm plankton net. The contents of the net were placed into an opaque 1-L bottle. Multiple tows were performed until the bottle was at least 75% full. Once back at the lab, some of the contents of the bottle were placed into a 500 mL beaker and were concentrated using a cup with a bottom consisting of 63 µm mesh material. This was repeated until all of the contents of the bottle had been concentrated to about 100 mL. A gridded Sedgewick-rafter cell was used to enumerate veligers in 1 mL of concentrated sample water using cross-polarized light (CPL) microscopy. This was repeated two more times and the average number of veligers / mL was calculated. The sample was further concentrated or diluted with filtered lake water until a concentration of 50-100 veligers / mL was obtained. A sample of veligers collected was preserved in ethanol for analysis of veliger stage and size distribution. The preserved sample of veligers was placed onto Sedgewick-rafter cells in 1 mL portions and investigated under CPL microscopy. When a veliger was found, a picture was taken and the length of the veliger was measured using SPOT software (Version 5.0.27, Diagnostic Instruments, Inc. Sterling Heights, MI).
Chemical Treatment

In a beaker, 24 mL of chemical solution was made by combining lake water (filtered through a 63 µm mesh) with the corresponding amount of dry or wet chemical to make 25 mL of solution for each concentration tested. There were two replicates for each concentration (0, 0.5, 1.0, 2% Virkon Aquatic and 0, 25, 50, 75, and 100% distilled white vinegar) and exposure time (2, 5, 10, 30 min for Virkon and 10, 20, 30 min for vinegar) combination for both chemicals tested. Exposure periods were chosen to include shorter and longer exposure periods than what was recommended. One mL of concentrated veliger water was added to each beaker using a pipette with the tip cut to prevent damage to the veligers. At the end of the exposure time, the contents of each beaker were poured into a veliger holding device and then rinsed with filtered lake water. The veliger holding devices were constructed by drilling a one-quarter inch hole in the cap of a 15 mL centrifuge tube, removing the screw-on cap and placing 63 µm mesh over the end of the tube and putting the cap back on, and finally cutting the pointed end of the centrifuge tube off. This design allowed the veligers to be constrained to a small area so that it would be easy to recollect them to estimate mortality while ensuring that they were exposed to aerated chemical-free water. The veligers were observed for mortality immediately following the lake water rinse using a Sedgewick rafter cell and cross polarized light microscopy. After each mortality assessment, the contents of the slide were rinsed back into the veliger holding device with filtered lake water. This was repeated every 24 h for 72 h following the initial mortality observation. Each veliger holding device was labelled with the chemical name, concentration, and exposure time and was placed into a recovery tank of filtered lake water that was lightly aerated with compressed air. Both samples for each concentration-exposure time were observed consecutively. The total number of all veligers for both observations was determined and if fewer than 30 veligers were recovered, another milliliter was observed from each replicate. In samples with more than 100 veligers, only the first 100 veligers observed were used for mortality assessment. Veligers were considered to be dead if they met any of the following criteria: no internal organs visible within the shell, internal organs leaking out of the shell, no movement of internal organs, and no movement of cilia (sensu Watters et al. 2013, Figure 5.1). Some chemicals have been shown to cause veligers to appear dead immediately following chemical exposure but after being placed into water without the chemical, the veligers are visually alive (Pucherelli et al. 2014).

Statistical Analysis

The average percentage of mortality was determined for each concentration and exposure time for each mortality assessment. The mortality data were transformed with an arcsin-square transformation due to the low replicate number and non-normal distribution (Zar 1996). An analysis of covariance (ANCOVA) was performed for each chemical to determine if the chemical concentration, the exposure time, or the interaction of the concentration and exposure time were significant factors of veliger mortality using SAS® (Version 9.3 SAS Institute Inc.,
Cary, NC). The ANCOVA was performed using the mortality data for the final mortality assessment in case there was any veliger recovery after removal from the chemical treatments. The level of significance for all tests was $\alpha = 0.05$.

Figure 5.1. Length-frequency of zebra mussel veligers removed from Otsego Lake, NY for distilled white vinegar and Virkon Aquatic toxicity experiments.
Results

The preserved sample of veligers consisted of 19 D-stage, 23 umbonal, and 9 pediveligers (Figure 5.2).

Figure 5.2. Veligers exposed to 0.5% Virkon Aquatic for 10 minutes. Note the open, empty shell on the left-most veliger and the crushed veliger on the right. Photo: Eric Davis

Distilled White Vinegar

Every concentration tested caused 100% mortality at every exposure period examined (Table 5.1). The control groups did not have greater than 46% mortality in the 72 h following the first mortality assessment. There were several instances where fewer than 30 veligers were assessed during this test. The shells on the veligers were dissolved by the vinegar so that they were not easily recognized when using CPL microscopy. At the 72 h post-removal mortality assessment, the difference was significant (F=8.64, p =0.0004), with concentration a significant factor in veliger mortality (p <0.0001).
Table 5.1. Average mortality (%) of zebra mussel veligers taken from Otsego Lake, NY at mortality assessments following removal from varying concentrations of distilled white vinegar.

<table>
<thead>
<tr>
<th>Exposure (minutes)</th>
<th>Concentration (% vinegar)</th>
<th>Mortality (%) of veligers at mortality assessment (h after removal from chemical treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>7.70</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>22.28</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>19.20</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*Virkon Aquatic*

*Virkon Aquatic* that was tested resulted in complete mortality for every exposure period (Table 5.2). The control group had an increase in average mortality in the 72 h period but no single sample exceeded greater than 50% mortality. There was no veliger recovery during the 72 h period of post-exposure mortality assessments for any treatment group. Chemical concentration was a significant factor of veliger mortality at the 72 h post-removal mortality assessment (p <0.0001) in the ANCOVA.
Table 5.2. Average mortality (%) of zebra mussel veligers taken from Otsego Lake, NY at mortality assessments following removal from varying concentrations of Virkon Aquatic.

<table>
<thead>
<tr>
<th>Exposure (minutes)</th>
<th>Concentration (% Virkon)</th>
<th>Mortality (%) of veligers at mortality assessment (h after removal from chemical treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>5.02</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>100</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>100</td>
</tr>
</tbody>
</table>

**Discussion**

The results of this test indicate that both Virkon Aquatic and distilled white vinegar can be used to disinfect watercraft exposed to zebra mussel veligers in a relatively short period of time. Both chemicals caused complete mortality with no veliger recovery by 72 h at low concentrations with exposure times of 2 min (Virkon) and 10 min (vinegar).

Distilled white vinegar was able to cause complete mortality in a much shorter time and at a lower concentration than that recommended by DiVittorio et al. (2012). It was recommended that undiluted white vinegar was used with a 20 min exposure period to allow for dreissenid mussel decontamination. In the current study, a 25% vinegar solution (75% lake water, 1.25% acetic acid) caused complete mortality with a 10 min exposure period. This would allow for a faster decontamination, with a lower cost than DiVittorio et al.’s (2012) recommendation, as the vinegar was diluted. Throughout the experiment, veligers that had been in the chemical solutions were difficult to identify during the post-exposure mortality assessments because they did not illuminate under the cross-polarized light. This may be due to the low pH of the treatments’
effect on the ability of the veligers to maintain calcium in their valves. This loss of calcium would cause the veliger to be incapable of continuing to grow and mature, eventually leading to mortality. The tolerance range for pH in adult mussels has been reported to be 6.5 (McCauley and Kott 1993) to 9.3 (Bowman and Bailey 1998). Zebra mussel veligers have been shown to have a pH tolerance range of 7.4 to 9.4 prior to settlement (Sprung 1993). In the most diluted treatment tanks during the test of distilled white vinegar on adult zebra mussels, the pH was less than 4 from time 0 h until 96 h (Davis et al. 2015). The pH of the vinegar solutions is not likely to change drastically if the solution was added to the bilge or livewell of a boat before being drained, unless the remnant water contains a high level of Ca+. This would allow for repeated use of the same solution once it has been made, allowing for decreased costs associated with disinfection using vinegar. The ability of the lowest concentration tested to cause complete mortality in the shortest exposure period may indicate the ability of an even more dilute concentration to be effective at veliger decontamination, or that these concentrations may be effective over even shorter periods of time.

Virkon Aquatic was effective at all concentrations at all exposure periods. This was in contrast to Moffitt et al. (2015), who reported found the 0.5% concentration of Virkon to be effective at exposures of 10 min but not at 5 min. It was also found that a 0.25% concentration was effective at causing complete mortality of veligers at exposure times of 15 and 20 min but not at 5 or 10 min (Moffitt et al. 2015). The 2.0% concentration was effective at a 5 min exposure period in that study as well (Moffitt et al. 2015). The veligers tested in that study were quagga mussels, which may explain the difference in results. Both the current study and the Moffitt et al. (2015) used room temperature (~20°C) chemical treatments. The active ingredients in Virkon Aquatic are potassium permonosulfate and sodium chloride (Dupont 2006). Fisher et al. (1991) found that potassium was toxic to zebra mussels at elevated concentrations. They also conducted further tests to investigate the mode of action of the potassium in zebra mussel mortality. They reported vacuolization of the epithelial cells of the gills of mussels exposed to elevated potassium levels and suggested the pathology was likely related to loss of fluid and/or electrolyte balance in the epithelial cells due to functional or structural changes in the plasma membrane (Fisher et al. 1991). There are test strips available that are fast-reacting so that the concentration of Virkon Aquatic can be quickly determined, allowing for reuse of the same solution as long as the concentration is maintained to provide adequate disinfection.

As suggested by Moffitt et al. (2015), further testing to investigate any change in the effectiveness of Virkon Aquatic at varying temperatures would be beneficial because veligers can be found in bodies of water at temperatures below 20°C. This could change the recommendations for concentrations and exposure periods by resource managers. A similar investigation of the efficacy of distilled white vinegar at varying temperatures would likely yield important data. When considering a chemical for use as a decontamination treatment, the cost is
often an important factor. The distilled white vinegar purchased for this study was $2.49 per gallon. If using the 25% vinegar dilution, four gallons of usable treatment solution could be made for $0.63 per gallon. If a lower concentration of distilled white vinegar was found to be effective at veliger decontamination, the cost of treatment would be less than $0.63/gallon. The Virkon Aquatic used for the study was purchased for $93.00 for a 10 pound tub (~4.5 kg). One tub of Virkon Aquatic makes 123 gallons of 1.0% solution or 246 gallons of 0.5%. This would mean a cost of $0.75 per gallon of 1.0% solution or $0.37 per gallon of 0.5% solution. Another important factor when investigating chemicals for decontamination use is their impact of the environment. The active ingredient in distilled white vinegar is acetic acid, which is an organic compound that rapidly breaks down into harmless substances in the environment (National Pollutant Inventory 2014). The active ingredients in Virkon Aquatic are inorganic oxidants that degrade into the environment as potassium and sulfate ions (DuPont 2006b). The remaining inorganic compounds in Virkon Aquatic break down into inorganic salts as levels that are not significantly higher than they naturally occur and the organic components are classified as readily biodegradable (DuPont 2006b).

Due to the low number of replicates that were performed during this study, another study that uses more replicates would allow for greater statistical analysis of the results and strengthen the ground from which decontamination protocols are decided. Also, an investigation of the effectiveness of the tested concentrations at shorter exposures would provide greater insight for the decision making due to complete mortality being caused at every exposure period tested.
CHAPTER 6: ABILITY TO USE A COMMERCIAL CARWASH TO REMOVE ATTACHED MUSSELS

Abstract
The use of hot-water spray is commonly recommended and employed by multiple government agencies in the United States to decontaminate watercraft with attached zebra mussels. The cost to construct a hot-water decontamination station at every public boat launch would be prohibitive. The ability of a pressure washer without heated water, similar to a commercial carwash, to remove attached juvenile and adult zebra mussels was examined. Clusters of attached mussels were removed from panels made out of an old canoe in an average of 197.1 ± 109.6 s. The ability of the pressure washer to remove attached mussels indicated that attached mussels could be removed without the need for heated water. The amount of time required to decontaminate an entire boat could take more than 7 hours and would likely be considered too long by recreational watercraft owners.

Introduction
Multiple natural resources agencies have started watercraft inspection and disinfection programs to stop the spread of the zebra and quagga mussels (Zook and Phillips 2015). Juvenile and adult mussels that have colonized the hull or other location of a watercraft can be extremely difficult to remove due to their byssal threads. The most common form of decontamination recommended is the use of hot water with high pressure (Comeau et al. 2011, DiVittorio 2015, Zook and Phillips 2015). This uses water heated to at least 60°C that is sprayed by a pressure washer (1,500 psi or higher) to kill and remove the mussels (Comeau et al. 2011, Jensen 2009, Morse 2009). While the use of hot water/high pressure has been shown to be effective, the cost to build a decontamination station that uses this technique can be problematic. A decontamination station would need to have electricity and plumbing in place, as well as a way to contain the waste water. Containing the waste water is important for two reasons. First, in case a mussel is removed by the high pressure of the spray before it is killed by the high temperature, the mussel could be able to survive and get into an uninfected body of water. Secondly, the reuse of the water may be important in some areas where there are water shortages or to reduce the amount of water being taken out of a water supply that could otherwise be used for human consumption. In addition to the cost to install plumbing and electricity, the equipment needed to build a hot-water spray station can cost in excess of $30,000 (Jensen 2009). In states that have hundreds or thousands of boat access locations, the construction of hot water/high pressure stations at every location is not possible. It has been shown that zebra mussels can be killed using hot water alone (39.26°C for mussels acclimated to 20°C, McMahon and Ussery 1995); however, there is little in the literature that has examined the use of unheated, high pressure water to remove attached mussels. The ability to remove attached mussels with high pressure water spray, without heating the water, would allow for decontamination of watercraft that is less
energy consumptive and relatively cheaper than using hot water with high pressure. Many towns have a commercial carwash where users pay to use unheated, somewhat high pressure water. If the water used for decontamination does not need to be heated to remove the mussels attached to a watercraft, it may be possible to recommend that watercraft owners go to a commercial carwash to remove mussels that are attached to their boat when either they remove their boat from the water or before launching at a new body of water. The pressure used at carwashes in the Lake Champlain area is between 1,000 and 1,300 psi (Lake Champlain Cooperative Boat Wash Program, 2014). Due to the slightly lower pressure used by many carwashes, a pressure washer that created pressure in that range was used to determine the effectiveness of removing attached mussels from panels used as surrogates for watercraft hulls. The hypothesis was that unheated water used in the pressure washer would remove all attached mussels.

### Methods

A fiberglass canoe was cut into fouling plates (28.3cm x 32.1cm). The plates were hung from a rack in Otsego Lake, NY at the Biological Field Station Main Laboratory from 30 August 2014 until 10 October 2015. When on the rack, the plates were suspended just below the surface of the water, where the depth was about 1.5 m.

Plates were examined for mussel colonization and a cluster of attached mussels were selected for removal testing. A picture of the area to be tested was taken with rulers placed on each plate so that mussel density could be calculated (Figure 6.1). The number of mussels was counted and recorded before testing began for each cluster. The cluster of mussels was then sprayed with the pressure washer (1,100 PSI, Excel Heated Pressure Washer Model 1003 VSWA) with a fan-shaped nozzle kept 10-15 cm (4-6 inches) from the plate with the heat turned off. The amount of time required to remove all mussels was recorded in seconds. This was repeated for a total of 30 replicates. The two plates with the most mussel colonization were tested separately to time the removal of mussels from the entire panel, to better mimic mussel removal on a larger scale, such as an entire boat hull. Fifty mussels were removed from plates in areas that were not selected for the pressure washer testing and were preserved in ethanol for size-distribution analysis. The maximum shell length was measured with a digital caliper (Model CD-6” CX, Mitutoyo Corp. Kawasaki, Japan).

The density of mussels per square meter was calculated for each cluster. The mean, standard deviation, and range were calculated for the number of mussels in a cluster, the density of mussels in the clusters, the removal time for the clusters, and the shell lengths. The linear correlation between the time to remove all mussels in a cluster and the number of mussels in a cluster was calculated. The linear correlation between the time to remove all mussels in a cluster and the density of mussels in the cluster was also calculated. All calculations were performed.
using SAS® (Version 9.3 SAS Institute Inc., Cary, NC) and the level of significance was set at \( \alpha = 0.05 \).

Results

The average cluster had 71.9 ± 45.7 mussels in it. The range of the number of mussels in a cluster was 23 to 207 mussels. It took an average of 197.1 ± 109.6 s to completely remove mussels from one cluster. The shortest amount of time to remove all mussels in a cluster was 18.67 s and the longest time to remove all mussels was 454.04 s (Figure 6.2). The average density of mussels was 27,020.24 mussels/m² in the clusters with a standard deviation of 17,740 mussels/m². There was not a significant linear correlation between the time to remove all mussels in a cluster and the number of mussels in a cluster \( (r = -0.12252, p = 0.5189) \) or between

Figure 6.1. Cluster of attached zebra mussels on canoe panel removed from Otsego Lake, NY with rulers used to calculate mussel density. Photo by Eric Davis.
the time to remove all mussels in a cluster and the density of mussels in a cluster ($r = 0.07585, p = 0.6904$).

![Figure 6.2. The time (s) to remove all attached zebra mussels from fiberglass canoe panels removed from Otsego Lake, NY.](image)

The first panel tested had 127 mussels with a density of 4,037.2 mussels/m². It took 282.92 s to completely remove the mussels on the panel. The second panel had 243 attached mussels with a density of 6,582.7 mussels/m². It took 817.46 s to remove all of the mussels attached to the second panel.

The 50 mussels that were measured for length-frequency had a mean length of 7.71 mm ± 4.37 mm. The range of mussel lengths was 3.31 – 16.65 mm. The mussels exhibited a bimodal distribution of shell length (Figure 6.3).
Figure 6.3. Length-frequency distribution for 50 zebra mussels attached to canoe panels removed from Otsego Lake, NY.

Discussion

The results of this test suggest that it is possible to remove attached mussels from watercraft with a pressure washer that has relatively low pressure and does not have high temperature water, such as those available at many commercial carwashes. The most heavily colonized panel took over 13 minutes for all mussels to be removed. To extrapolate this to cover a one-panel high strip on each side of a 16-foot boat would suggest a total time greater than 7 hours to wash. The removal for attached mussels from a large area could take more time than a watercraft owner may be willing to spend on pressure washing their watercraft.

The average amount of time to remove attached mussels was lower than that needed in another study that used 1,500 PSI pressure (Wong et al. 2014). That study broke down the results by the density of mussels. It took $472 \pm 178$ s to remove high density clusters of zebra mussels ($6,068 \pm 530$ mussels). It took an average of $41 \pm 14$s to remove low density clusters of zebra mussels ($1,068 \pm 1,091$ mussels). However, when looking at the overall average for time to remove all mussels, there was not a significant difference between that study and the current study although different pressures were used. This may be due to a slight difference in methodology when spraying the mussels with the pressure washer. In the current study, the
nozzle was kept 4-6 inches from the mussels while the nozzle was kept 12 inches from the mussels by Wong et al (2014). That study also tested removing mussels with 3,000 psi. The time to remove mussels was significantly lower at 3,000 psi than what was needed at 1,100 psi in the current study. The pressure used for removing mussels and the density of mussels were significant factors in determining the time needed to remove all mussels in Wong et al. (2014).

The plates used in this study were placed in the lake in late summer and allowed to sit for over a year before the test was conducted. This allowed some mussels to attach to the plates in the fall of 2014 and overwinter on the plates. Additionally, during the summer 2015 spawning season, more mussels colonized the plates. This resulted in two distinct size classes of mussel attached to the plates. This led to the non-normal distribution of shell length. In many northern states, boats are not overwintered in the lake due to ice formation therefore any mussels that have settled during that years’ spawning season would be killed by desiccation and/or freezing over the winter when the watercraft is stored out of water. The likelihood of a watercraft arriving at another body of water with mussels attached to it would be low unless it has been sitting in a mussel infested lake for an extended amount of time (i.e. 2-3 weeks) during the spawning season and is being moved in the latter part of the spawning season or after the spawning period. This would suggest that removing attached mussels from watercraft using high pressure with unheated water would be possible in places where ice cover does not allow for boats to be overwintered in bodies of water and when the colonization of mussels is limited to a relatively small area of the boat.
CHAPTER 7: LIVEWELL FLUSHING TO REMOVE ZEBRA MUSSEL VELIGERS

Abstract
The prevention of the spread of the zebra mussel is of great concern in many places in North America. The cost of constructing wash stations that provide hot water at high pressure is often limiting to their application as a decontamination method. The ability to use a garden hose to flush veliger-rich residual water from the livewell of a boat was examined. Veligers were found in samples taken from the livewell discharge after 5 min of flushing had been conducted. Although the flushing was not found to be completely effective, more than 90% of all veligers that were found during testing were collected during the first 150 s of flushing.

Introduction
The zebra mussel continues to spread across North America almost three decades after its first documented presence in the Great Lakes (Benson 2014, Carlton 2008). This continuous expansion is often attributed to the overland transport of trailered watercraft that contain larval mussels, called veligers (Johnson et al. 2009). Watercraft decontamination protocols have been developed to address watercraft that are suspected of harboring zebra mussel veligers (DiVittorio et al. 2012). The recommended and most used form of decontamination is hot water/high pressure spray (Comeau et al. 2011, DiVittorio et al. 2012, DiVittorio 2015, Zook and Phillips 2015). This method uses water heated to temperatures of 140°F applied for 10 s in an area to adequately kill zebra mussels (Morse 2009, DiVittorio et al. 2012). Due to the cost associated with constructing a hot water spray station (i.e. over $30,000 [Jensen 2009]), an alternative method to remove any veligers that remain in a trailered watercraft that is less expensive could result in a greater decontamination effort from the general public. Under the assumption that veligers that remain in the livewell or bilge area of a watercraft can be introduced into bodies of water once the boat is launched, the veligers that remain in those areas should be able to be removed by flushing those areas out with “clean” water. In the present study, the ability of a garden hose to flush the contents of a watercraft’s livewell within a reasonable length of time was investigated.

Methods

Veliger Collection
Zebra mussel veligers were collected from the top 3 m of the water column of Otsego Lake, NY using horizontal tows (about 750 m per tow) with a 63 µm plankton net from 25 August to 02 September. The contents of the net were placed into a 1 L bottle. Multiple tows (10-15) were performed until the bottle was at least 75% full, then they were taken to the main laboratory at the Biological Field Station. Once back at the lab, the contents of the bottle were concentrated down to 100 mL of liquid using a cup with a bottom of 63 µm net material. A
Sedgewick-rafter cell was used to count the number of veligers in 1 ml of concentrated sample water using cross-polarized light (CPL) microscopy. This was repeated two more times and the average number of veligers per milliliter was calculated. Veligers were collected in the morning and were only used for replicates during the same day.

Livewell Flushing

The livewell of a 16-foot aluminum johnboat was used for all livewell flushing tests. The plug was placed into the livewell and filled with ~22 L of veliger-free well water. Then enough concentrated veliger solution was added to bring the veliger density in the livewell to 10-200 veligers/L. After waiting for 30 s to let the veligers distribute throughout the livewell, the plug was removed and the livewell was drained. After 3 min, a funnel and PVC pipe drain were placed under the livewell drain. At the terminal end of the drain pipe, a 45° elbow directed contents of the pipe towards the ground. A piece of 3” PVC was set below the end of the drain pipe with a 63 µm mesh inside a coupler, across the pipe, so that the netting remained tight. The livewell was then flushed using a sprayer using a FIMCO HighFlo Gold Series pump (Model 5277981; 60 PSI) that ran on a 12-volt battery, equipped with a garden hose attachment on the end that was set on the “Stream” setting for a total of 7 min. During that time, every 30 s the 3” pipe piece was removed and another 3” pipe with filter was placed under the drain pipe until the 5 min point. The final piece of 3” pipe was placed under the drain pipe for the final 2 min of flushing and for 3 min of draining following the flushing. Each of the pipe pieces were triple back-flushed into a 250 mL beaker. The contents of the beaker were then poured into a veliger holding device (VHD). The VHDs were constructed by drilling a one-quarter inch hole in the cap of a 15 mL centrifuge tube, removing the screw-on cap and placing 63 µm net material over the end of the tube and putting the cap back on, and finally cutting the pointed end of the centrifuge tube off. This design allowed the veligers to be constrained in a small area so that it would be easy to recollect them for preservation. The VHDs were placed in a test tube rack so that only about 2 mL of water was inside the holding device. A pipette was used to collect the contents of the VHD and place them into a 15 mL centrifuge tube. Then 3 mL of 70% ethanol was added to the centrifuge tube to preserve the contents for analysis at a later date because the mortality of veligers was not important, only if they were present in the livewell following the flushing attempt. Each tube was labelled with the replicate number and the interval in the flushing time. A 25% distilled white vinegar solution was then poured into the livewell and allowed to soak for 10 min (this solution caused the shell of veligers to be dissolved during other testing (Davis et al. 2015, Chapter 5) and would make it so veligers left behind by one replicate were not counted for the following replicate). Following the soaking period, the livewell was flushed twice by filling the plugged livewell with ~22 L of water and then draining. A total of thirty replicates were performed over the course of 8 days from 25 August to 02 September. Any equipment that could have had contact with veligers during a replicate was rinsed three times with veliger-free well water before the next replicate was started.
Preserved Sample Analysis

The bottom 1 mL of sample in the centrifuge tube was removed with a pipette with the tip cut and placed into a gridded Sedgewick-rafter cell. The sample was then examined under cross-polarized light microscopy and the number of veligers present was counted and recorded. This was repeated two more times for each preserved sample. Between sample enumerations, the Sedgewick-rafter cells and cover slips were rinsed with veliger-free water and dried.

Statistical Analysis

The number of veligers observed in each time interval sample was converted to the percentage of the total veligers observed in the replicate. Because the test was performed as a series of time intervals, the nonparametric Kendall’s tau correlation coefficient was calculated to investigate the relationship between the time of flushing and the percentage of veligers removed using SAS (Version 9.3 SAS Institute Inc., Cary, NC). The level of significance was set at $\alpha=0.05$.

Results

There were an average of 440.7 veligers identified per replicate (Table 7.1). The first 30 s after flushing began had the greatest percentage of total removed veligers (Table 7.2, Figure 7.1). There was a significant correlation between the time after flushing began and the percentage of veligers that were removed (Kendall’s tau correlation coefficient = -0.632, $p < 0.0001$, $N = 330$).
Table 7.1. Number of veligers observed in samples taken at intervals during livewell flushing and average number of veligers observed per interval.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Concentration (veligers/L)</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
<th>210</th>
<th>240</th>
<th>270</th>
<th>300</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>42</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>127</td>
<td>14</td>
<td>18</td>
<td>19</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>624</td>
<td>133</td>
<td>68</td>
<td>84</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>222</td>
<td>68</td>
<td>105</td>
<td>47</td>
<td>5</td>
<td>9</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>18</td>
<td>26</td>
<td>146</td>
<td>118</td>
<td>139</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>394</td>
<td>77</td>
<td>35</td>
<td>12</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>200</td>
<td>374</td>
<td>104</td>
<td>20</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>6</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>200</td>
<td>44</td>
<td>42</td>
<td>45</td>
<td>24</td>
<td>20</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>200</td>
<td>321</td>
<td>84</td>
<td>120</td>
<td>51</td>
<td>19</td>
<td>10</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>200</td>
<td>94</td>
<td>85</td>
<td>61</td>
<td>43</td>
<td>17</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>200</td>
<td>307</td>
<td>122</td>
<td>63</td>
<td>44</td>
<td>39</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>200</td>
<td>137</td>
<td>91</td>
<td>67</td>
<td>67</td>
<td>15</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>200</td>
<td>281</td>
<td>132</td>
<td>93</td>
<td>54</td>
<td>34</td>
<td>10</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>200</td>
<td>17</td>
<td>9</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>200</td>
<td>284</td>
<td>72</td>
<td>82</td>
<td>18</td>
<td>24</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>16</td>
<td>200</td>
<td>219</td>
<td>48</td>
<td>77</td>
<td>69</td>
<td>25</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>200</td>
<td>265</td>
<td>54</td>
<td>82</td>
<td>51</td>
<td>15</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>18</td>
<td>200</td>
<td>225</td>
<td>107</td>
<td>36</td>
<td>14</td>
<td>13</td>
<td>9</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>19</td>
<td>200</td>
<td>188</td>
<td>103</td>
<td>58</td>
<td>33</td>
<td>33</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>200</td>
<td>167</td>
<td>73</td>
<td>17</td>
<td>39</td>
<td>32</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>21</td>
<td>200</td>
<td>324</td>
<td>48</td>
<td>77</td>
<td>32</td>
<td>34</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>22</td>
<td>200</td>
<td>100</td>
<td>83</td>
<td>25</td>
<td>48</td>
<td>18</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>23</td>
<td>200</td>
<td>258</td>
<td>104</td>
<td>62</td>
<td>37</td>
<td>20</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>24</td>
<td>200</td>
<td>253</td>
<td>63</td>
<td>79</td>
<td>37</td>
<td>37</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>25</td>
<td>200</td>
<td>275</td>
<td>58</td>
<td>31</td>
<td>43</td>
<td>27</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>26</td>
<td>200</td>
<td>163</td>
<td>74</td>
<td>50</td>
<td>50</td>
<td>29</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>27</td>
<td>200</td>
<td>325</td>
<td>102</td>
<td>33</td>
<td>65</td>
<td>16</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>28</td>
<td>200</td>
<td>224</td>
<td>103</td>
<td>32</td>
<td>26</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>29</td>
<td>200</td>
<td>283</td>
<td>50</td>
<td>22</td>
<td>36</td>
<td>36</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>30</td>
<td>200</td>
<td>159</td>
<td>47</td>
<td>80</td>
<td>44</td>
<td>38</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 7.2. Average percentage of veligers observed at intervals after livewell flushing began.

<table>
<thead>
<tr>
<th>Time after livewell flushing began (s)</th>
<th>Average percentage of veligers observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>49.0%</td>
</tr>
<tr>
<td>60</td>
<td>16.3%</td>
</tr>
<tr>
<td>90</td>
<td>12.9%</td>
</tr>
<tr>
<td>120</td>
<td>9.2%</td>
</tr>
<tr>
<td>150</td>
<td>5.9%</td>
</tr>
<tr>
<td>180</td>
<td>1.5%</td>
</tr>
<tr>
<td>210</td>
<td>0.8%</td>
</tr>
<tr>
<td>240</td>
<td>1.0%</td>
</tr>
<tr>
<td>270</td>
<td>1.1%</td>
</tr>
<tr>
<td>300</td>
<td>0.9%</td>
</tr>
<tr>
<td>600</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

Figure 7.1. Veligers identified in each interval as a percentage of the entire replicate at intervals after livewell flushing began.

**Discussion**

The correlation between the time of flushing and the percentage of veligers observed indicated that as time of flushing increased, the percentage of veligers observed decreased significantly. Even with this correlation, the results of the study suggest that a 5 min interval is not sufficient to remove all veligers from the livewell of a boat. There were an average of 4.8 veligers observed in the final sample of each replicate. Only one replicate had 0 veligers in the final sample while 4 replicates had 10 or more veligers in the final sample. Veligers remaining in the livewell after 5 min of flushing could be transported and introduced into another body of water. A longer flushing period could result in the removal of more veligers but is not likely to
be performed by watercraft owners. Jensen (2009) found that the majority of boat owners (69%) were willing to spend 5 min to get their boat washed. Only 26% of boat owners were willing to spend 10 min at a boat wash station (Jensen 2009). Under natural conditions, veliger densities typically are below 40 veligers/L (Gerstenberger et al. 2011); however, peak densities of 200 veligers/L of zebra mussel veligers have been found in western Lake Erie (Garton and Haag 1993). While the concentration of veligers tested here represent the highest concentration seen during an entire spawning season, which may only occur for a period of a few days, it is likely that watercraft would encounter lower densities of veligers which may be more easily removed by flushing.

During the first 150 s of flushing, greater than 90% of veligers were removed. Over 90% of all veligers observed for an entire replicate were observed on average in samples from 150 s of flushing or shorter. This short period of flushing can remove a large proportion of the veligers that are left in the livewell, which can greatly reduce the spread of veligers when no other decontamination method is available for use. A reduced number of veligers can help slow the spread of zebra mussels because it decreases the likelihood that veligers will settle close enough to each for reproduction to occur.

The effectiveness of livewell flushing could likely be greatly increased if used in combination with a chemical treatment that causes mortality to zebra mussel veligers. Distilled white vinegar was found to cause complete veliger mortality with a 10 min exposure (Davis et al., Chapter 5). A vinegar treatment prior to livewell flushing could result in complete removal of veligers by rendering them immobile by killing them and all allowing the flowing water to sweep all of the veligers out of the livewell. Examining the effectiveness of a vinegar treatment followed by livewell flushing would provide valuable data that could significantly impact watercraft decontamination protocols.
REFERENCES


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

National Pollutant Inventory. 2014. Acetic acid. Taken from: \url{http://www.npi.gov.au/resource/acetic-acidethanoic-acid}


Padilla DK, Chotkowski MA, Buchan LAJ. 1996. Predicting the spread of zebra mussels (	extit{Dreissena polymorpha}) to inland waters using boater movement patterns. 	extit{Global Ecological Biogeography} 5:353–359.


OCCASIONAL PAPERS PUBLISHED BY THE BIOLOGICAL FIELD STATION (cont.)


No. 43. The Upper Susquehanna watershed project: A fusion of science and pedagogy. Todd Paternoster. 2008.


No. 47. The state of Hatch Lake and Bradley Brook Reservoir, 2015 & a plan for the management of Hatch Lake and Bradley Brook Reservoir. Jason E. Luce. 2015.


Annual Reports and Technical Reports published by the Biological Field Station are available at:
http://www.oneonta.edu/academics/biofld/publications.asp