The Use of Elevated pH to Reduce the Risk of Release of Select Invasive Mollusk Species from Vessel Ballast and Bilge Water

A Thesis

Presented in Partial Fulfillment of the Requirements for the

Degree of Master of Science

with a

Major in Fishery Resources

in the

College of Graduate Studies

University of Idaho

by

Amber L. Barenberg

Major Professor: Christine M. Moffitt, Ph.D.

Committee Members: Frank M. Wilhelm, Ph.D.; Barnaby J. Watten, Ph.D.

Department Administrator: Lisette Waits, Ph.D.

April 2015

Authorization to Submit Thesis

The thesis of Amber Barenberg, submitted for the degree of Master of Science with a major in Fishery Resources and titled "The Use of Elevated pH to Reduce the Risk of Release of Select Invasive Mollusk Species from Vessel Ballast and Bilge Water," has been reviewed in final form. Permission, as indicated by the signatures and date given below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor:

_____ Date _____
Christine M. Moffitt, Ph.D.

Committee Members:

_____ Date _____
Barnaby J. Watten, Ph.D.

_____ Date _____
Frank Wilhelm, Ph.D.

Department Administrator:

_____ Date _____
Lisette Waits, Ph.D.

Discipline's College Dean:

_____ Date _____
Kurt S. Pregitzer, Ph.D.

Final approval and Acceptance

Dean of the College of Graduate Studies:

_____ Date _____
Jie Chen, Ph.D.

Abstract

I tested the efficacy of aqueous solutions of sodium hydroxide, NaOH, and calcium hydroxide, $Ca(OH)_2$, (pH 11-12), to cause mortality in New Zealand mud snails (NZMS - *Potamopyrgus antipodarum*), quagga mussels (*Dreissena bugensis)*, and Asian clams (*Corbicula fluminea)*. Static toxicity tests were conducted in freshwater with all three species, and a limited number of trials were conducted in brackish seawater (15 ppt) with quagga mussels. The quagga mussel was most sensitive to elevated pH, followed by the NZMS, and then the Asian clam. Time to mortality was most rapid at warm temperatures compared to cold temperatures, and early life stages were more sensitive than advanced life stages. My results provide strong evidence that elevated pH would be a successful disinfection tool against aquatic invasive mollusks in ballast and bilge water.

Acknowledgements

I would like to thank my major professor Dr. Christine Moffitt for her help, encouragement, and guidance with this project. I would also like to thank the members on my committee, Drs. Barnaby Watten and Frank Wilhelm for their expertise. Special thanks to the US Geological Survey and U.S. Fish and Wildlife Service for providing funding for this project. I would also like to acknowledge and thank the Hagerman National Fish Hatchery, Willow Beach National Fish Hatchery, and the Idaho State Department of Agriculture for providing me with test specimens for my project.

I am in debt to the following people; Kelly Stockton, Justin Shearer, Mindy Torres, Laura Hughes, Charlie Withers-Haley, Tasha Venable, Jenna Davis, Juan Carlos Ortiz-Perez, Kate Wilcox, Mary Frances Babrowicz, Brandon Snow, Taylar McClure, Marilina Gartiez, Tom Woolf, Matt TenEyck, Lubia Cajas de Gliniwicz, and Zach Penny for their ideas and assistance with laboratory procedures, data analysis, and field work. I would also like to thank the Center for Research on Invasive Species and Small Populations Research Experience for Undergraduates (CRISSP REU), the Doris Duke Research Fellowship, and the HOIST programs for providing talented interns to assist with research.

I am very appreciative to my parents Scott and Karen Barenberg, my siblings Brian, Kimber, Travis, and by dogs Shasta and Ginger for their love, support, encouragement, and patience. Thanks to my friends in the Boise and Moscow areas for their encouragement and belief in my abilities. I appreciate the University of Idaho for providing me the opportunity to further my education in science and pursue my Master's Degree in fisheries resources.

Dedication

This thesis is dedicated to my wonderful supportive parents Richard Scott Barenberg and Karen Barenberg and also in loving memory of Shasta and Ginger, two of the best friends I have ever had the privilege to know. Thank you for all of your unconditional love throughout the years. You are greatly missed.

Table of Contents

List of Tables

List of Figures

Introduction

There are about 50,000 non-native species in the United States (Pimentel et al. 2005). Of these non-native species, 4,300 are considered invasive (Corn et al. 1999, cited by U.S. Fish and Wildlife Service 2012). Invasive species come in many different shapes and sizes and include species from both terrestrial and aquatic ecosystems. Dr. David Pimentel estimated that damage from invasive species cost the U.S. about 137 billion dollars annually (Wakefield and Faulds 2003). Some of the most notorious aquatic introductions that have occurred in the U.S. include the Asian carps (*Hypophthalmichthys nobilis, Mylopharyngodon piceus, Ctenopharyngodon idella, and Hypophthalmichthys molitrix*) and the quagga mussel (*Dreissena bugensis*) and the zebra mussel (*Dreissena polymorpha*). Costs and damages to the U.S. from aquatic invasive species alone are estimated to be 9 billion dollars with fish causing the most damage (5.4 billion), followed by the quagga and zebra mussels (1 million), the Asian clam (1 million), and others (1.8 billion) (Wakefield and Faulds 2003). The economic costs and disruptions caused by aquatic invasive species have created challenges for aquatic resource management agencies and water users. The focus of this thesis is on selected species of aquatic invasive mollusks. Mollusks encompass several different types of invertebrates, which include snails, mussels, slugs, and octopus. The experiments summarized in this thesis include studies with an invasive snail, mussel, and clam.

Many vectors are associated with assisting in the establishment of invasive species and include wading boots, motor vehicles, recreational boats and trailers, live wells and bilges (Johnson et al. 2001; Waterkeyn et al. 2010; Stockton and Moffitt 2013). Other vectors include the aquarium trade, release of aquarium pets, hitchhiking on the feet and in the fur of

animals, and even intentional introductions (Yan et al. 2001; Semmens et al. 2004; Frisch et al. 2007; Vanschoenwinkel et al. 2008; Bruce et al. 2009). Mechanisms for global transport of aquatic invasive species include the food trade and ballast tanks of cargo ships (Williams et al. 1988; Yan et al. 2001).

Ballast water discharge is one of the primary mechanisms by which new species are introduced to coastal and fresh water ecosystems like the Great Lakes (Ricciardi and MacIsaac 2000). Ballast tanks are comprised of individual compartments located inside a cargo ship that can be pumped full with water and discharged individually to maintain ship balance when cargo is being loaded and unloaded and also to maintain structural integrity during voyage (UNEP, no date.; IMO 2015) (Appendix 1 and 2). Some ballast tanks are capable of holding millions of liters of water (Ricciardi and MacIsaac 2000). The United Nations Environmental Programme (UNEP) (n.d.) has estimated that about 14 billion tons of ballast water are transported globally every year and that ballasts can contain as much as 10,000 different species. However, ballast water is not the only culprit, the bilge and live well water inside recreational boats has also assisted in dispersing aquatic species over land across states (Johnson et al. 2001). As the demands for goods increases globally, the number of ships and potential invaders will likely increase. It is important to have ballast discharge regulations and boat check stations in place that protect local waters from foreign invaders.

Management agencies need ways to protect non-infested water bodies and to contain and prevent the spread of invasive species from existing sites of infestation. For many agencies, the primary focus of prevention is targeting the vectors such as boats and ballast tanks or other large volume vectors most responsible for the transport of invasive species into non-

infected waters. Vehicle and boat check stations are used to assess and disinfect infested recreational and small commercial boats with hot pressurized water (Rothlisberger et al. 2010). Options of disinfection include chemical and mechanical processes (Nielson et al. 2012a, 2012b) such as the use of bleach, copper, bromine, and ozone (Tsolaki and Diamadopoulos 2010). While many of these chemical options can be convenient and effective, they can be expensive, harmful to the environment and human health, and difficult to dispose of (Tsolaki and Diamadopoulos 2010; Moffitt et al. in press). Mechanical options of disinfection include manual removal, temperature shock, mechanical filtration, ultraviolet light exposure, and vehicle confiscation and isolation for several weeks (Tsolaki and Diamadopoulos 2010). While these options are not as harmful to the environment or human health as the chemical methods, they can be expensive, labor intensive, have lower success rates, and be difficult to scale up for large volumes of water in cases of ship ballast and hatchery trucks (Tsolaki and Diamadopoulos 2010).

To help protect non-infected water bodies from harmful organisms there are national and international discharge regulations in place that are implemented and enforced by the U.S. Coast Guard and International Maritime Organization (IMO). On June 21, 2012, the United States Coast Guard implemented new regulations for the discharge of ballast water. These regulations require seagoing vessels to incorporate a ballast water treatment system by the year 2016. Ballast water discharge must contain < 10 organisms per m^3 of ballast water for organisms ≥ 50 µm, and < 10 organisms per mL of ballast water for organisms < 50 µm (U.S. Federal Register 2014). Due to the expense and hazards of chemical options and difficulty in

scaling up mechanical options for larger volumes of water, there is need for disinfection options that are effective, environmentally compliant, economically feasible, and safe to use.

Sodium hydroxide, NaOH, and calcium hydroxide, $Ca(OH)_2$, also known as lye and hydrated lime respectively, are two chemicals that hold promise as disinfectants, and meet the above criteria (Starliper and Watten 2013; Moffitt et al., in press). These chemicals can be used to elevate the pH of water. High pH means the solution is alkaline and that there is a high concentration of negatively charged hydroxyl ions (OH^-). In studies by Claudi et al. (2012), elevated pH was shown to inhibit settlement of dreissenid veligers and cause tissue damage to adult mussels by affecting their ability to osmoregulate. Previous studies conducted by the Great Ships Initiative (GSI), in cooperation with the USGS, and located at the University of Wisconsin-Superior in Superior, Wisconsin, have shown these chemicals are capable of reducing the risk of release of potential invasive species from ballast tanks. The GSI staff provided results on the pH stability of different dosages and the effective dosage on selected resilient algae and zooplankton species, the residual toxicity of the chemicals to sensitive non-target species, as well as chemical degradation in the presence of different environmental parameters such as organic material. In pH stability tests with $Ca(OH)_2$ and NaOH they found that high pH levels (12.2) were more stable than pH levels of 11.5, and that high dosages of elevated pH (12.2 and 12) were more effective at killing selected species of green algae and zooplankton and significantly lowered cyst hatching than pH levels of 11.5. Even in the presence of sediment, burrowing species still experienced significantly higher mortality in tests compared to controls at the higher pH levels. The studies also showed that sensitive non-target species had 90-100% survival after pH levels had been diluted (TenEyck and Cangelosi

2009, TenEyck et al. 2011). The success of the bench scale studies of NaOH warranted further research by GSI to include both a land-based study and shipboard study. The land-based study examined NaOH and its ability to disinfect ballast successfully without interruption, reduce the number of live organisms compared to controls, be successfully neutralized by carbon dioxide (CO_2) and compliant with discharge regulations, and contain no residual toxicity after a 2-3 day holding time (Cangelosi et al. 2011). Like the bench scale tests, the land-based test showed promising results. The neutralization with CO_2 was successful and met discharge standards. The shipboard trial with NaOH on the M/V Indiana Harbor (bulk carrier) found treated tanks had lower survival of target organisms ≥ 50 μm and organisms' ≥ 10 and < 50 μm than control tanks. For organisms < 10 μm one of the treated tanks had higher levels of live coliform bacteria than the controls. The authors highlighted the need for further tests to provide conclusive results (Cangelosi et al. 2013).

In addition to causing high mortality rates in selected species and having limited effects on sensitive species after being diluted, these chemicals have several other properties that make them appealing as potential disinfection tools. NaOH and $Ca(OH)_2$ can be manufactured in many forms including a pelleted and powder form and both chemicals are soluble in water. Very little product is needed to raise the pH of fresh water from neutral to a strong alkaline solution (OECD SIDS 2002; U.S. Department of Health and Human Services 2002; ClearTech Industries Inc. 2009). In addition alkaline solutions do not negatively affect the structural integrity of steel, which is important for the ballast tanks and associated plumbing of ships (Department of Energy 1993).

NaOH and $Ca(OH)_2$ are relatively inexpensive on a commercial scale compared to other disinfection chemicals such as Virkon® aquatic. The use of NaOH (50% bulk solution) mixed to 12 pH would cost approximately 2 times more than a treatment based on use of $Ca(OH)_2$. Markestad (2010), estimated that 28,682 L (43,853.3 kg) of 50% NaOH solution would be needed to treat a ballast tank system (bulk freighter) that holds an average of 11.3 million gal. Cost of the NaOH is then $6,139. This does not include the cost of neutralization.

The high alkaline solution created with NaOH and $Ca(OH)_2$ can be easily neutralized with carbon dioxide gas yielding sodium carbonate and calcium carbonate respectively, which in low doses are environmentally harmless compounds (Cangelosi et al. 2013; Starliper and Watten 2013). Sodium carbonate, for example, is used domestically to soften water and calcium carbonate is a non-toxic environmentally beneficial substance, aiding in the formation of shells, skeletons and coral reefs (EPA 2006; Solvay Sustainable 2011, 2012).

Neutralizing ballast tanks with CO_2 appears to be economically feasible. CO_2 costs $0.15/Kg (Barnaby Watten emails dated 30 January 2015). In the report by Markestad (2010) the dose rate of 0.67 g of CO_2 for 1 L of ballast water was sufficient to neutralize a 12 pH tank to below 9 pH, which is considered acceptable for overboard discharge. To neutralize 45,425 m^3 ($4.5425X10^7$ L) ballast tank, simply multiply $4.5425X10^7$ L by the dose rate (0.67 g) for a total of 30,434.7 Kg of CO_2. Finally multiply 30,434.7 Kg by $0.15 (cost per kg of CO_2) for a total of $4,500.00 to neutralize 45,425 m^3 of ballast water. The cost of CO_2 treatment is then $4,298 for the 11.7 million gallon example and the total cost of treatment is then $10,427.

Due to the promising nature of the NaOH and $Ca(OH)_2$ compounds, I chose to examine the effect of elevated pH, range of 11 to 12, on three important aquatic invasive mollusks known for their prolific nature and their resilience to chemical and environmental stressors. Those species are the New Zealand mudsnail (*Potamopyrgus antipodarum*) (NZMS), the Asian clam (*Corbicula fluminea)*, and the quagga mussel (***Dreissena bugensis)***.

New Zealand Mudsnail (NZMS)

NZMS were first reported in the United States in Hagerman Idaho in 1987 (Bowler 1991). Since then, they have been detected in the Snake River and the surrounding western states of WA, OR, NV, MT, UT, and WY (Bowler and Frest 1996). The initial vector of establishment in the state of Idaho was likely the movement and sale of fish and trout eggs (Bowler 1991). A separate introduction by way of ship ballast water allowed the NZMS to also become established in the Great Lakes (Zaranko et al. 1997). NZMS are small (6-7 mm) and can reach densities higher than 500,000 individuals/m^2 in their introduced habitats (Winterbourn 1970; Hall et al. 2003). NZMS are parthenogenetic and ovoviviparous and a single snail can release up to 230 young annually (Lassen 1979; Bowler 1991; Richards et al. 2004). NZMS are especially difficult to kill due to their reproductive potential and operculum that serves to protect the snails from predators, toxic environments and desiccation (Richards et al. 2004; Nielson et al. 2012b; Stockton and Moffitt 2013). An adult NZMS can survive without water for 24 h and up to 50 days in a damp environment and can survive transit through the GI tract of fish (Winterbourn 1970; Bruce et al. 2009). NZMS can also tolerate a wide range of environmental conditions. For example, they can tolerate short term exposures to salinity up to 33 ppt and have been active in salinity up to 17 ppt (Winterbourn 1970; Cheng and LeClair

2011). NZMS prefer temperatures of approximately 15°C but can tolerate temperatures between 0-34°C although freezing temperatures that last for more than 4 days are generally lethal to most of the population, reducing survival to 1.8% of the population. (Winterbourn 1969; Cox and Rutherford 2000; Cheng and Leclair 2011; Moffitt and James 2012). In locations of the Silver Creek watershed in Idaho, Moffitt and James (2012) found no presence of NZMS in areas that experienced temperatures of 0°C and below.

When NZMS infest a new area they can have many impacts on the economy and ecosystem. They can directly compete with endangered and native benthic organisms for food and space (Kerns et al. 2005; Strzelec 2005; Hall et al. 2006). NZMS have the ability to consume up to 75% of the primary productivity and can physically prevent other organisms from occupying the area due to their high density (Hall et al. 2003). Like other invasive mollusks, NZMS can have an indirect impact on higher trophic levels, such as fish, by removing available food sources. NZMS have also been found to be of little nutritional value when consumed by fish, although fish will readily consume them (Bruce and Moffitt 2010). In fish diet studies, those fed NZMS had lower body weight and condition compared to fish fed a normal diet (McCarter 1986; Vinson and Baker 2008). The shells of NZMS are difficult for fish to digest, and in some cases the NZMS can survive passage through the digestive tract. This provides a potential dispersal mechanism to new areas where they can establish new populations (Bondesen and Kaiser 1949; Haynes et al. 1985; Bruce et al. 2009). In addition to having low nutritional value, NZMS are also hosts to parasites, such as trematodes, bacteria and viruses, which can be passed to water fowl or fish (McArthur and Featherston 1976; Krist and Lively 1998; U.S. Fish and Wildlife Service 2004).

Asian Clams

Asian clams were first detected on the west coast of North America in the early 1920's (McMahon 1982, 1991). It is speculated that they were brought to the United States as a food item by Chinese immigrants (Counts 1981). Since their introduction, Asian clams have been reported in nearly every state in the Unites States with the exception of Montana, North Dakota, Main, Vermont and Alaska (Foster et al. 2012). The Asian clam has many characteristics that make it a successful invader. Individuals reach between 50-65 mm in length and densities as high as 20,000 individuals/m^2 (McMahon 1999). Asian clams have a life span between 1 and 7 years and produce approximately 69,000 young per individual each breeding season after reaching maturity (Aldridge and McMahon 1978; McMahon 2002). Asian clams are hermaphrodites, and can cross fertilize as well as self-fertilize (Kraemer et al. 1986). They brood their young and release them fully developed (McMahon 1999). The Asian clam can filter and pedal feed, the latter of which allows them to continue growing when resources in the water column become scarce (Way et al. 1990; Boltovskoy et al. 1995; Hakenkamp et al. 2001). Individuals can filter up to 1.3 L of water per hour, meaning that at high densities, they filter and remove particles from a large volume of water (Way et al. 1990; Silverman et al. 1995). Asian clams can tolerate a wide range of environmental conditions. For example, Evans et al. (1979) and Balcom (1994) reported survival at temperatures between 2-36°C and in salinity up to 24 ppt. Under low environmental oxygen or other unfavorable environmental conditions, Asian clams can survive inside their closed shells. During valve closure the Asian clam can reduce its metabolism to 10% of original standard metabolic rates and can survive off of the oxygen inside the shell for up to 10-12 h before

toxins begin to accumulate in their tissues (Ortmann and Grieshaber 2003). This valve closure characteristic is one of the reasons Asian clams made ideal test subjects for our toxicology tests.

Asian clams can compete directly with native mollusks and other invertebrates for food and space due to their high filtration rates and abundance (Yeager et al. 2000; Karatayev et al 2003; McMahon 1999). In some cases it is estimated that Asian clams can remove as much as 70% of the phytoplankton biomass from the water column resulting in an estimated 75% loss of biomass of zooplankton in areas of high clam abundance (Lauristen 1986; Pigneur 2014). The Asian clam removes nutrients from the water column and concentrates waste materials as pseudo feces on the benthos (Hakenkamp and Palmer 1999). Better sunlight penetration and a more fertile benthos cause macrophyte, algal, and bacterial blooms that can result in massive die off of Asian clams as a result of poor oxygen when the bloom die off (Phelps 1994). These die offs also increase ammonia concentrations, which can negatively affect other benthic organisms (Scheller 1997). The hard shells left behind by Asian clams can serve as a substrate for the establishment of other organisms including other invasive species such as zebra mussels, which require hard substrates to attach and grow (Lewandowski 1976; McMahon 1999; Gutierrez et al. 2003).

Asian clams have no byssal threads that attach them to substrates, however, the Asian clam is still a nuisance for waste water treatment plants, power plants, and irrigation systems (McMahon 1999) because large accumulations of clams and dead shells can clog pipes and often require managers to dewater the system and remove them manually. Pimentel (2000)

estimated the costs of control, equipment replacement and repair because of Asian clams costs approximately $1 billion annually.

Quagga Mussel

Quagga mussels, first found in the United States in the Great Lakes in 1989, are believed to have originated from the Southern Bug River in the Ukraine via discharged ballast water of transoceanic ships (Mills et al. 1996; Ricciardi and MacIsaac 2000). Currently quagga mussels have spread to most states in the U.S. east and have recently been found in some states in the west, mostly confined to the south (Benson et al. 2014). Presently, the northwest has no known infestations of quagga mussels (Benson et al. 2014). However, Warziniack et al. (2013) found that should quagga mussels become established in the Columbia River Basin, it could increase electricity costs from hydropower facilities by $23.6 million as a result of increased maintenance costs.

Quagga mussels are small (2-4 cm) and adults typically attach to both soft and hard sediments, industrial structures, and to each other with byssal threads (Dermott and Munawar 1993; Coyne et al. 1997; Benson et al. 2014). Because of the byssal threads quagga mussels can reach densities as high as 54,000 individuals per m^2 (Turner et al. 2011). Unlike the NZMS and the Asian Clam, quagga mussels are dioecious and broadcast spawn (Ram et al. 1996). A single quagga mussel female is capable of producing up to one million eggs annually (Keller et al. 2007).

Water quality parameters, especially the presence of suitable calcium, are important in determining the likelihood of infestation of quagga mussels into new locations. Jones and

Ricciardi (2005) and Whittier et al. (2008) have found that quagga mussels do well at calcium levels ≥ 28 mg Ca/L Quagga mussels appear to have lower environmental tolerances than NZMS and Asian clam. Quagga mussels can survive in temperatures between 0-25°C and are able to spawn at 4.8°C (Spidle et al. 1995; Roe and MacIsaac 1997; Karatayev et al. 2007). The quagga mussels tolerate salinities less than 5 ppt and prefer pH levels between 7.4 and 9.4 (Sprung 1993; Spidle et al. 1995).

The major economical consequence of quagga mussels is biofouling of any structure in contact with infested waters including industrial structures, pipes, screens, navigational buoys, and boats (Claudi and Mackie 1993; Connely et al. 2007). Biofouling of dams is another major concern. An assessment by DeBruyckere and Phillips (2014) determined the risk of establishment of quagga mussel on 75 dams in the northwest based on parameters such as water body size and access, presences of boat ramps, the number of fishing and angling tournaments, as well as environmental parameters such as dissolved oxygen, calcium concentrations, and pH. Of the 75 dams assessed, 18 were considered to be high risk for quagga mussel establishment and 21 were considered medium risk. If quagga mussels become established in the Columbia River Basin, management officials fear they could interfere with the passage of fish via fish ladders and juvenile bypass facilities at dams (IEAB 2013). There is also fear that they could be hosts to pathogens that are harmful to fish and create areas of "dead zones" within streams and rivers as a result of waste production (IEAB 2013). Quagga mussels can reach high densities inside inflow pipes and seal off these structures rendering them useless. High densities can also affect native benthic invertebrates. Quagga mussels can use the shells and bodies of native mussels and crustaceans as places to anchor and grow. As

the number of mussels on the surface of the native increase it can eventually prevent movement, feeding, and eventually suffocate the individual (Schloesser et al. 1996; Ricciardi et al. 1998). At the base of the byssal thread attachment bacteria can grow and respire causing industrial structures to decompose (Mackie and Claudi 2009).

Quagga mussels also negatively affect recreation; large numbers of shells, which are very sharp, accumulate on beaches during massive die offs and deter tourists and locals from visiting. Boaters may also become reluctant to put their vehicles in infested waters due to strict cleaning guidelines and the potential for biofouling on the hulls and propellers, which can increase drag and ruin engines (Oregon Sea Grant 2010). Quagga mussels can also reduce fishing opportunities by altering the flow of energy in food webs. Their selective filter feeding removes phytoplankton, which reduce food available to zooplankton populations. Smaller populations of zooplankton affect larval fish and planktivore populations and in turn piscivores (Pace et al. 1998; Strayer et al. 2004; Hinderer et al. 2011). Quagga mussels can also negatively affect fish populations by colonizing spawning grounds and degrading feeding areas (Turner et al. 2011).

The estimated costs of mitigation, research, structure replacement, cleaning, and management planning in regards to aquatic invasive mollusks are in the millions of dollars annually (Pimentel et al. 2005). Prevention is often a much cheaper option than managing an established population (Keller et al. 2008; Leung et al. 2002). It is important to find alternative methods of disinfection that are successful, economically feasible, and environmentally compliant.

Objectives and Predictions

The objectives of this study were to determine and model the mortality of three invasive mollusks species of concern in static exposure trials of aqueous NaOH or $Ca(OH)_2$ at pH range of 11 to 12 at different test temperatures (12-20°C). Tests were conducted on large mature adults and early life history stages of each of the three species.

Based on peer-reviewed literature of the physical attributes and environmental tolerances of each species, I predicted that the adult NZMS would require the longest time to 100% mortality followed by the adult Asian clam and then adult quagga mussels. I also predicted that the early life stages of each species would have a shorter time to 100% mortality than the adult life stage and that warmer temperatures would have a shorter time to 100% mortality than tests conducted at colder test temperatures. I also predicted that the time to mortality would be similar regardless of the chemical used if all other testing conditions remained identical.

Methods

Study Sites and Test Organisms

All toxicology trials were conducted between 2012 and 2014.

Experiments with NZMS and Asian clams at the University of Idaho

Tests with New Zealand mudsnails (NZMS) and Asian clams were conducted in the College of Natural Resources at the University of Idaho, Moscow. Test populations of Asian clams

and New Zealand mud snails were held in a secured temperature controlled isolation room in the fisheries wet laboratory and maintained on a natural photoperiod cycle for the latitude of Hagerman National Fish Hatchery. Experiments were conducted in the isolation room or in other laboratory space.

The NZMS for tests conducted at the University were obtained from springs at Hagerman National Fish Hatchery, Hagerman, Idaho and express shipped to the University of Idaho via overnight courier. Asian clams were collected from the Bruneau River Idaho, and transported to the University in coolers or express shipped overnight. All organisms were carefully packed in wet towels, placed in plastic bags, and placed in a cooler with ice packs prior to shipping.

Upon arrival the bags with snails or clams were opened and placed in the containment room to acclimate to 15°C for at least one hour. After acclimation, test organisms were rinsed of sediments, and divided into plastic containers filled with de-chlorinated well water (domestic well water filtered through carbon to remove any free chlorine) at 15°C. The NZMS were divided into several 1.8 L plastic containers (< 500 individuals/container) filled with 1 L of de-chlorinated well water. All of the water in cultures of NZMS was exchanged every other day. The Asian clams were kept in 5 L containers with supplemented air supply and each container was given a pinch of 50:50 mixture of pulverized brine shrimp and algae pellets daily. The water in each container was exchanged daily. To ensure specimen viability, fresh test organisms were obtained from source locations every 2-3 weeks.

New Zealand mudsnail neonates were obtained from several glass beakers containing brooding adults or from 1.8 L plastic containers from the confinement room harboring adults. Glass beakers or containers were placed under a dissecting microscope and live neonates were removed with a transfer pipette and placed into the testing beakers. Asian clam veligers were obtained by inducing adults to release them (Appendix 3). After 24 h, the veligers were removed and placed into a separate labeled 1.8 L container aerated with air stones. Veligers were collected over several days for up to one week from containers with adults, after which any new veligers released from adults would be placed into a new labeled holding container.

Experiments with NZMS and quagga mussels at Willow Beach National Fish Hatchery

Tests with quagga mussels and some tests with adult NZMS (Trial S2 and S3) were conducted at Willow Beach National Fish Hatchery (WBNFH), White Hills, Arizona located on the Colorado River 18 km downstream from Hoover Dam. Experiments were conducted inside the laboratory of the juvenile fish rearing facility. For these trials, experimental animals were collected from hatchery raceways by hand and did not require transport or shipping. Several adult mussels and NZMS (2 mm) were placed into a large 13 L bucket filled with approximately 10 L of raceway water. Organisms were acclimated for 2 h to test temperature, and sorted by size for the experiments.

Quagga mussel veligers were obtained from the overflow of the head box of raceway B8 at WBNFH with a 35-µm mesh plankton net (Sea-gear Corporation, Melbourne, FL) with a cod jar. The plankton net was left in place for 30 minutes, afterwards the net was removed and water in cod jar was filtered through a 500 µm (US-35; 0.500 mm) mesh stainless steel sieve

to remove large debris and algae. The filtrate was stored in a 1 L Nalgene bottle for 1 h to allow contents to settle. To concentrate veligers for testing the contents of the Nalgene bottle were filtered through a 90-μm screen and backwashed into a clean 1.8 L container with a gentle stream of water from a plastic squeeze bottle.

Preparations of Test Solutions and Controls

All test solutions were prepared in 2 L increments using an Erlenmeyer flask or beaker. Solutions were then combined and stored in a capped 20 L polyethylene carboy to limit exposure to atmospheric CO_2. Tests with NaOH were prepared by grinding pellets (Fisher Brand NaOH certified A.C.S. grade, Lot number 060432 and 994932) with a mortar and pestle, to a fine powder. The powder was slowly administered to fresh aged well water (water that had been left un-capped for ≥ 24 h and was chlorine free) (de-chlorinated), raceway water, or half-strength sea water to achieve the target pH (approximately 0.68 g/L for fresh well water and 2.22 g/L brackish raceway water). Test solutions of $Ca(OH)_2$ (Powder USP/FCC, lot number 120311), also mixed in 2 L increments, were prepared and then filtered through a 9.0 diameter Whatman #4 filter paper under vacuum to clarify the solution before being stored in a capped 20 L polyethylene carboy.

Several trials with quagga mussels were conducted in 15 ppt seawater to simulate brackish water conditions and examine survival of the target species if they encountered sea water. The brackish water was created by adding artificial sea salts (Crystal Sea Marinemix Marine Enterprises International Inc. Baltimore, Maryland) to de-chlorinated well water or raceway water. The target pH and salinity levels were confirmed with a calibrated YSI 556 multi probe

(YSI Corporation, Yellow Springs, Ohio) or an UltraBASIC pH/mV meter (Denver Instrument, Bohemia, New York). The carboy and test solution contents were acclimated to desired test temperature for 24 h before testing. Water for controls was de-chlorinated well water or raceway water and was also acclimated to test temperature for 24 h prior to testing.

Quality Assurance for Equipment and Measuring Water Quality

All tools and containers used for experiments were decontaminated before and after trials by soaking in 10% bleach solution for at least 10 min. followed by neutralization in sodium thiosulfate and then air dried. Monitoring equipment was rinsed thoroughly in deionized water and air dried.

Air and water temperatures for each experiment were monitored with Hobo temperature loggers (Onset Computer Corporation, Cape Cod, Massachusetts) set to record once per hour that were placed in cups or beakers of de-chlorinated water to mock experimental containers. Other parameters including pH, conductivity, salinity, and dissolved oxygen were measured at each time interval in each test replicate for every trial using a YSI 556 multi-meter probe and a Hach HQ30D fitted with a IntelliCAL LDO101 Rugged Luminescent/Optical Dissolved Oxygen Probe (Hach, Loveland, Colorado).

Experimental Design and Procedures for Conducting Tests

Experiments were conducted using static exposures to aqueous NaOH or $Ca(OH)_2$ with solution renewal every 24 h, which helped to maintain water quality through the duration of the experiment. Tests were conducted at room temperature (19-26°C) for all three species.

Trials with quagga mussels were also conducted at a cool temperature (15-17°C). In addition, quagga mussels were exposed to elevated pH created with NaOH in 15 ppt sea water and also brackish water without elevated pH at the same time at the same test temperature (room temperature), to determine if this species was sensitive to salt solutions (Table 1).

The early life history stage and different sizes of larger individual stages of each species were tested. Trials with NZMS were conducted on individual's ≥ 2 mm in length (adults) and with neonates (< 1 mm). Asian clam adults were ≥ 16 mm in length and veligers were 200-300 µm long. Tests with quagga mussels were conducted with individuals' ≥ 24 mm long, individuals 6-15 mm, and veligers 70-200 µm. For the purpose of this paper, distinguishing between the two sizes of fully formed quagga mussels were based on size measurements not reproductive status of the species. "Adult" was used to describe the larger individuals' (≥ 24 mm long) and "juvenile" the smaller sized individuals (6-15 mm). Microscopic stages were called "neonate" (NZMS) and "veliger" (Asian clam and quagga mussel).

Twenty-three trials were conducted at a pH of 12 (Table 1). In trials with $Ca(OH)_2$, five trials were conducted at a pH of 11.2, 11.4, and 11.8. Seven trials were conducted with NZMS, three of which were with adults and four with neonates. Eight trials were conducted using Asian clams, three of which were with adults and five with veligers. Thirteen trials were conducted using quagga mussels, of which six were with adults, three with juveniles, and four with veligers. The veliger trials included two in NaOH and two in $Ca(OH)_2$. Within each chemical one trial was conducted at cool temperature and the other at room temperature.

Twenty-four trials were conducted in fresh water and four trials were conducted in brackish water (Table 1). For trials conducted with quagga mussels in brackish water, two trials were conducted with adults and two with juveniles. For experiments with adults, after exposure to brackish (15 ppt) NaOH, adults were allowed to recover in fresh de-chlorinated well water for 48 h. In one of the trials the juvenile mussels recovered in 15 ppt brackish water and the controls for this trial were 15 ppt brackish water. In the other trial the juveniles recovered in fresh water and controls for this experiment were fresh water.

Trials were reported by target pH, where the range of measures fell within ±0.10 pH units of the mean. However, in one trial with Asian clams (trial C3) the pH range changed by 0.32 (12.02-11.7) but was included into the target pH of 12. Exposure times ranged from seconds to days, depending on the life history stage and test temperature. Sampling intervals were arranged at log intervals to facilitate response modeling. At each sampling interval, 2 to 5 replicate containers and at least one non-exposed control were removed to examine mortality. Replicates contained between 2-10 adult sized organisms or ≥ 10 individuals for trials with veligers and neonates.

Randomly chosen organisms to be used in tests were removed from holding systems or raceways and placed into containers or buckets with source water from the holding systems or raceways. To reduce mortality caused by rapid change in temperature, test organisms in containers or buckets of source water were allowed to acclimate to test temperatures 2–24 h prior to toxicology testing. Tests with Asian clams and NZMS were all conducted at room temperature. Replicates for adult sized NZMS consisted of 50 mm diameter stainless steel tea balls (500 µm mesh), each of which contained 10 individuals, that were placed into 1.8 L

containers containing the test solution. Each 1.8 L container holds between 7-8 tea balls. Asian clams and quagga mussels were placed into 1.8 L plastic containers or 150 mL glass beakers each of which served as a test replicate. Due to their larger size and high filtration rates, 10 or fewer adult or juvenile size clams or mussels were placed into each replicate. Tests with neonates and veligers were conducted in 150 mL glass beakers with 10 individuals per replicate.

To begin a trial with quagga mussels and Asian clams, acclimation water was drained from containers and then the containers were filled rapidly with the solution. For trials with NZMS, tea balls with NZMS were lifted and drained from acclimation containers and placed into a container of test solution. All test containers were filled entirely and covered to prevent exposure to atmospheric CO_2. For tests lasting longer than 24 h, the solution in test replicates and controls was exchanged daily. Loss of organisms was prevented by securing the organisms in one corner of the container and carefully decanting the old solution into a waste bucket. During the solution exchange, organisms were exposed to air for < 5 sec.

At each exposure interval with adults, 2 to 5 replicates were removed from containers with test solution, while 1-5 replicates were removed from controls, rinsed in a three bath system of aged water to remove the test chemical, and then placed into containers with aged well water for recovery for 48 h. For veligers and neonates, individuals were removed from test solutions by filtering through a 65-µm mesh sieve, after which they were washed with 120 mL of de-chlorinated well water, and then back-washed into a recovery beaker containing aged well water where they were kept for 24 h. All recovery containers were inspected to account for all organisms. Recovery containers were maintained at the same temperature as

the tests. For Asian clams, supplemental aeration was provided for 48 h after being removed from the test solution. In all recovery containers, the water was replaced every 24 h to ensure constant water quality.

The response variable in all trials was the number or proportion of individuals that died, while the independent factor was exposure time in minutes or hours. I used a dissecting microscope to assess mortality of veligers/neonates and adults. The number and status (alive or dead) of each individual was determined by assessing movement. Those not moving were probed with a dissecting needle to attempt to elicit a response. Snails that were responsive to probing or observed actively crawling around the recovery container were scored as alive. For quagga mussels and Asian clams, organisms that were responsive to probes or closed and difficult to pry open were considered alive. Organisms that were open and unresponsive, or showed signs of decomposition were scored as dead. At the end of the trials all organisms were placed in plastic bags and frozen until incineration.

The number of live, dead, and total individuals in each test and control replicate was recorded. Data were ranked based on the criteria provided by the US Environmental Protection Agency which states that tests for acute toxicity (tests lasting $\leq$ 96 h exposure) using adult invertebrates should not have control mortalities exceeding 10%, while tests for chronic toxicity (tests lasting > 96 h exposure) should not have control mortalities exceeding 20% (USEPA 2001, USEPA 2002). For tests with egg and larval stages, control mortality should not exceed 30% to accurately assess response in treatments (USEPA 1985).

Mortality responses of each trial were graphed with scatter plots to inspect data. Statistical analyses of the data included use of probit regression models with normal distribution when adequate response time intervals were available to fit a model. Probit regression models were used to predict the median lethal time to 99% mortality (LT99). Probit regression models were constructed to include covariates of test replicate and trial along with the variable of time of exposure. Trials were considered highly accurate if the time was significant, test replicates were not different, and confidence intervals could be estimated. If multiple trials were conducted with the same organisms at similar test conditions and the trials were not significantly different from each other, the trials were combined to produce a conservative best model estimate of LT99.

When the number of treatment intervals was too few to allow probit regression predictions, the frequency of live and dead between treatments or between treatment and controls was evaluated using categorical analysis and chi-squared or Fisher's exact frequency tests to compare response within and between tests at the same time interval. For comparisons between different pH, test temperatures, or time exposures, the proportion dead was also analyzed with generalized linear models with the response variables percent dead. Finally, in cases with inadequate data for statistical models, survival curves were used to estimate the minimum time to mortality. All statistical models were analyzed using SAS 9.3 (SAS Institute, Cary North Carolina).

Results

NZMS

For NZMS in $Ca(OH)_2$ at room temperature, pH ranged from 11.96-12.04, while the pH in the controls ranged from 7.94-8.05 (Table 2, trial S1). The estimated median LT99 for adult NZMS exposed to $Ca(OH)_2$ (target pH 12) was 65.2 h (95% CI = 50-94 h) (Table 3, Figure 1). Mortality response within replicates for each sampling interval of trial S1 did not differ significantly ($P = 0.95$). Time of exposure was a significant factor on mortality response ($P = <0.0001$).

In trials with adult NZMS in NaOH at room temperature, the pH ranged from 11.9-12.01 and 11.75-11.87 in test solution replicates for trials S2 (12 pH) and S3 (11.8 ph), respectively (Table 2). In control replicates the pH ranged from 7.92-8.35 for trial S2 and 8.19-8.07 for S3. These trials showed a faster time to mortality than tests in $Ca(OH)_2$. Even though trial S2 and S3 had a different target pH, the mortality response between trials was not significantly different ($P = 0.63$). Trials were combined for probit modeling. Replicates were not significantly different ($P = 0.92$) and time was significant ($P = 0.0001$). The estimated LT99 was 39 h, 95% CI = 31.9-60.7 h (Table 4, Figure 2).

In trials with neonate NZMS in NaOH at room temperature, pH in test replicates ranged from 12.06-11.94 (S4), 12.05-11.97 (S5), 12.08-12.00 (S6), and 12.08-11.88 (S7). Mortality was very rapid for neonates. Mortality response between trials was not significantly different ($P = 0.32$). Replicate was not significant ($P = 0.97$), but time was significant ($P = <0.0001$). The

LT99 was 5.6 min with a lower and upper CI of 4.1 to 8.6 min respectively (Table 4; Figure 3).

Asian clams

The range in pH for test solution replicates in the trial with Asian clam adults in $Ca(OH)_2$ (Trial C1) at room temperature was 11.78 to 11.93, while the control pH ranged from 7.91 to 7.94 (Table 5). The target treatment pH was 11.8 pH. The probit model converged but 95% CI were not produced. The LT99 was 274 h. Covariates of replicate (0.17) and time (0.21) were not significantly different (Table 6). For all time intervals (150, 226, 275 and 342 h), treatment mortalities were significantly higher than controls (Fisher's exact all $P = \leq 0.01$), supporting the hypothesis that pH 11.8 was effective (Table 7).

Asian clam veligers were more sensitive to elevated pH than adults. The mean pH of the test solution in trial C5, C6 and C8 was 11.7, 11.96, and 11.82, respectively (Table 5). The mean pH of test solutions in trials C4 and C7 was 11.4 and 11.46, respectively (Table 5). Veligers mortality was highest in the 11.4 (C4 and C7) and 12.0 (C6) pH treatment compared to the other treatments tested (Table 8).

In the 11.2 pH treatment 71-100% mortality was observed in the longest time interval (90 min), suggesting that veligers need to be exposed to this pH for a longer period of time to achieve 100% mortality in all test replicates. In the two 11.4 pH treatments, 100% mortality in all replicates was achieved at 60 and 90 min in the first and second experiments, respectively. Veligers in the pH 11.8 treatment were less sensitive at the 60 min time interval and required 120 min to achieve 88-100% mortality (Table 8; Figure 4). A longer exposure to 11.8 pH is

required to achieve 100% mortality. At pH 12.0, veliger mortality occurred in all test replicates by 60 min (Table 8; Figure 4).

Mortality response of veligers was analyzed at the 60 min time interval of all experiments using a GLM (Mortality = Treatment) to determine if there was any significant difference between treatment solutions. Mortality response of veligers in pH treatments was significantly different ($F_{3,12}$ = 202.9, P = <0.0001) except for those tested in 12.0 pH and 11.4 pH (Table 9). Mortality response of veligers was similar in 12.0 pH and 11.4 pH but significantly higher than the other pH treatments. The same procedure was run for the different pH treatments at the 90 min time interval and mortality response was not significantly different between treatments ($F_{2, 6}$ = 1.74, P = 0.25) due to high mortality in all experiments (Table 10).

A Fisher's exact test was used to test mortality response at identical time exposures in experiments with veligers to determine if mortality in test replicates was significantly higher than control replicates. For the trial at 11.2 pH, treatment was significantly higher after 75 min (Fisher's exact P = <0.0001) but there was no significant difference in mortality response in the 60 min time interval (Fisher's exact P = 0.075) (Table 11). In pH 11.4, mortality was significantly higher in all time intervals in both trials in the treatment replicates (Fisher's exact all P = <0.0001) (Table 12). Trials at 11.4 pH had similar mortality response in test treatments at the 60 min time interval (Fishers exact P = 0.37) suggesting that the mortality between trials was similar (Table 13). For the trials conducted at pH 11.8 and 12.0 pH, response in test treatments was significantly higher than control treatments at all time intervals (Fisher's exact all P = <0.0001) (Tables 14 and 15).

The two trials with adult Asian clams in NaOH at room temperature (trial C2 and C3) with target pH of 12.0 were not significantly different (P = 0.79), and the probit model for these trials fit a prediction with an estimated median LT99 of 215 h and 95% CI = 189-251 h (Table 16; Figure 5).

Quagga mussels

Quagga mussels experienced rapid mortality response to elevated pH. In the trial conducted with adult mussels in $Ca(OH)_2$ (trial Q1) at room temperature, pH ranged between 11.95 and 12.02 (Table 17). The estimated median LT99 was 7.9 h, 95% CI = 4.8-47.9 h (Table 18).Mortality response for time was significant (P = 0.03) and there was no significant effect of replicate (P = 0.91). Mortality in the treatments was significantly higher than in the controls at all time intervals (Fisher's exact all P = <0.0001) (Table 19).

Veligers were more sensitive to elevated pH in solutions of $Ca(OH)_2$ than adults. Mortality response of test replicates between the veliger trial conducted at room temperature (Trial Q11) and the veliger trail conducted at cooler temperature (Q13) were analyzed at both the 2 min and 5 min sampling intervals to determine if there was a difference between the two temperatures. Mortality response in the test replicates of the room temperature trial was significantly higher at both 2 and 5 min than veligers tested at cooler temperatures (Table 20). The estimated median LT99 for the veliger trial at room and cooler temperature was 6.7 and 6.3 min, respectively (Table 21; Figure 6 and 7). At both experimental temperatures, mortality in treatment replicates was higher than the controls at all time intervals (Table 22).

Mortality responses in trials with adult mussels in NaOH at room temperature conducted in May and November of 2012 were not significantly different (P = 0.11; Table 23). Because there was no difference in mortality response, trials were combined and probit model estimate of LT99 was 33.7 h; 95% CI = 25-53 h. In May, the observed minimum time to 100% mortality was 18 h and mortality in the test replicates was higher than in the control replicates starting at 14 h exposure (Table 24). In November, the observed minimum time to mortality was 42 h and mortality response was significantly higher in treatment replicates than controls at 15 h (Table 24). In the trial with adult quagga mussels in NaOH at cool temperature (Trial Q4), 100% mortality was observed in all replicates at 72 h of exposure. Only two individuals were tested in each replicate for controls and treatments. There was high survival in almost all treatment replicates at all sampling intervals except for the last sampling interval (72 h), in which significantly higher mortality occurred in the treatment replicates than in the controls (Fisher's exact P = 0.04) (Table 25).

Mortality in juvenile quagga mussels occurred more rapidly than for adults in NaOH at room temperature (Trial Q7). The estimated LT99 for the juvenile trial conducted in fresh water at a mean pH of 11.98 (Table 17) was 13 h (95% CI 10-17 h) (Table 26, Figure 8). Mortality response for time intervals (P = <0.0001) and replicate (P = 0.0005) was significant. Because mortality between replicates differed, mortality response between the treatment replicates was compared with the response in controls at each time interval to determine if treatment was effective in causing mortality. Mortality response was significantly higher in treatments than in controls beginning at the 4 h time interval (P = 0.0391) (Table 27).

The quagga mussel veligers showed the most rapid mortality relative to adults and juveniles tested in NaOH at room temperature. Observed lethal time to 100% mortality occurred at the 10 min time interval and mortality responses in treatment solutions was higher than controls at all sampling time intervals (Table 28). For the trial at the cool temperature (Trial Q12), mortality was significantly higher than controls at all sampling intervals (Fisher's exact P = ≤0.001) (Table 28). The estimated LT99 for veligers tested at cooler temperatures was 21 min; 95% CI = 16-29 min (Table 29, Figure 9). Mortality response of the treatment replicates between the trials was analyzed at shared intervals (5, 10, 20 minutes) to determine if temperature affected mortality. Mortality response was more rapid in the room temperature trial at the 10 min interval (P = <0.0001) but not at the 5 and 20 min intervals (Table 30).

Quagga mussel adults tested in brackish water experienced rapid mortality regardless of the presence of elevated pH. The mean pH for the brackish tests in elevated pH was 11.9 generated via NaOH, and the neutral pH it was 8.43, while in the controls the pH ranged from 8.39 to 8.49 (Table 31). Because mortality (P = 0.07) between trials was greater than $\alpha = 0.05$ trials were combined in the probit model and produced an estimated LT99 of 14.3 h; 95% CI = 11.5-19 h (Table 32; Figure 10).

The mortality of juveniles in the brackish solution was more rapid than for adults (*P* = <0.0001) at the 2 h sampling interval (Table 33). The observed minimum time to 100% mortality for juveniles was approximately 2 h, while for adults it was 10 h. Juveniles were also more sensitive to brackish solutions than to fresh water solutions of elevated pH. For the juveniles that recovered in fresh water, mortality was higher in treatments solutions than in the controls at 2 h sampling interval and later (Table 34). In contrast, for the juveniles that

recovered in 15 ppt brackish water, all replicates in both controls and tests had 100% mortality, indicating that exposure to 15 ppt seawater can be lethal to juvenile mussels. The mortality response in test replicates between the two juvenile trials at 1 h was significantly different (Fisher's exact P = 0.03) with more rapid mortality occurring in the trial that recovered in brackish water (Table 35). However, a 2 h time exposure regardless of recovery treatment caused 100% mortality.

Discussion

Elevated pH of 11.2 to 12 successfully caused mortality to three species of invasive mollusks. The results in this study were similar to those by TenEyck et al. (2009, 2011) who found that long exposure times to test solutions and high pH levels were effective in killing resilient organisms and resulted in more rapid LT99 over shorter exposure times and lower pH. In tests with $Ca(OH)_2$ at 25°C, the mortality of the green alga (*Selenastrum capricornutum*) was 22% (pH 11.5) and 0% (12 pH) after 48 h exposure. Fresh water rotifers (*Brachionus calyciflorus*) exposed for 24 h to pH 11.5 and 12 had 73% and 0% survivability, respectively. Daphnids (*Daphnia magna*) and Copepods (*Eucyclops* sp.) did not survive when exposed to pH 11.5 and 12. TenEyck et al. (2011) found that cysts of the rotifer *Brachionus calyciflorus* were the most difficult to kill with high pH. Fifty-three and 39% of cysts hatched after a 48 h exposure to pH 11.5 and 12, respectively. Green algae had higher survival in exposures to NaOH than to $Ca(OH)_2$. Survival rate was 95% in the 11.5 pH and 9% in 12 pH after a 48 h exposure to NaOH. In exposures to $Ca(OH)_2$ survival was 22% in 11.5 pH and 0% in 12 pH, however pH levels dropped in this trial from pH 12 to 11.77 and from 11.5 to 8.5 after 48 h exposure. Both Daphnids and Copepods had the same survival rate in NaOH as $Ca(OH)_2$ with no survival in

both 11.5 pH and 12 pH after 24 h exposure. Both fresh water rotifers and their cysts were more sensitive to NaOH than to exposure of $Ca(OH)_2$. No rotifers survived exposure to pH 11.5 and 12 after 24 h. However, 5% of cysts remained viable after 48 h exposure to pH 11.5 and 12 (TenEyck et al. 2009; 2011).

Even though NaOH and $Ca(OH)_2$ were both successful in killing invasive mollusks, the mortality response differed between reagents. NaOH was more lethal than $Ca(OH)_2$ in tests with Asian clams and NZMS. However, quagga mussel veligers and adults were less tolerant of $Ca(OH)_2$ than NaOH. This differential sensitivity among mollusks to NaOH and $Ca(OH)_2$ has implications when choosing a disinfectant to treat ballast waters. While $Ca(OH)_2$ is less expensive than NaOH, it is less effective when targeting NZMS and/or Asian clams.

It is important to consider that seasonal changes can affect the mortality response in mollusks. Claudi et al. (2012) also conducted a study using elevated pH with NaOH to control quagga and zebra mussels. Their tests were conducted at 13°C and they evaluated the settling rate, mortality response, and corrosive potential of NaOH. In separate tests, one in May and one in October with adult zebra mussels at a pH of 12 pH 99% mortality occurred at 24 h in May, while in the October trial 90% mortality only was reached at 120 h. Claudi et al. (2012) noted that the experiment conducted in May was likely compromised by harsh winter conditions, which resulted in weaker organisms at the start of the experiment. My results were similar to the studies conducted by Claudi et al. (2012). In my trials with quagga mussels at 20°C in NaOH an earlier time to 100% mortality was observed in the trial conducted in May (18 h), than in the November trial (42 h). Based on our experiments and the ones conducted by Claudi et al. (2012) it is likely that time of year can affect the response to the solution. Zebra

and quagga mussels tested in the spring are most likely weaker than those tested in the fall because in the spring, organisms are likely recovering from exposure to freezing temperatures and reduced food availability resulting from the winter season.

Claudi et al. (2012) also investigated the effects of elevated pH on the corrosion rate of carbon steel, copper, and stainless steel. Claudi et al. (2012) found that corrosion rates of elevated pH solutions on carbon steel and copper were lower than controls, but that there was no difference between control and elevated pH on the corrosion rates of stainless steel. This supports the idea that elevated pH can help maintain the structural integrity of ballast tanks especially if constructed from carbon steel or copper.

The Asian clam appears to have a unique ability to withstand harsh conditions much longer than NZMS or the quagga mussel. Quagga mussel adults tested in my experiments had the shortest time to mortality of all three species followed by the NZMS adult and lastly the Asian clam adults. NZMS can use the operculum to withstand long periods of desiccation (Winterbourn 1970) or to protect itself from harsh environmental conditions. The soft body of Asian clams is protected by the mantel lobes as well as the shell, which is thick and armored compared to shells of quagga mussels or NZMS. Asian clams also have a higher lethal temperature (36°C) than the NZMS (34°C) or the quagga mussel (25°C) and this may have factored into the low mortality response that was detected for Asian clams in experiments conducted at room temperature. Bidwell et al. (1995) found that Asian clams had a longer time to mortality than Zebra mussels (*Dreissena polymorpha*) when exposed to the molluscicide DGH/QUAT at 20 to 25°C. They reasoned that the zebra mussels may have spent a longer time siphoning the molluscicide then the Asian clam or that the Asian clams

just have an advanced ability to tolerate stress associated with exposure. However, the adult specimens (Asian clams, New Zealand mudsnails, and quagga mussels) were all closed prior to solution being administered and quagga mussels and Asian clams did not siphon nor did New Zealand mudsnails move in our experiments while exposed to test solution. Asian clams can reduce their metabolic rate by 90% during valve closure and remain in an aerobic state for up to 9 h (Ortmann and Grieshaber 2003) temporarily avoiding the lethal effects of pollutants until the accumulation of toxic end products. Ortmann and Grieshaber (2003) noted that once closed it was not uncommon for an Asian clam to remain closed for between 10 and 12 hours. Unstressed and in cooler temperatures, Asian clams can remain closed for an even longer period of time. Ortmann and Grieshaber (2003) observed Asian clams to remain closed for over 1 week at 5°C. Matthews and McMahon (1999) looked at the effects of temperature on extreme hypoxia (O_2 < 3% of full air saturation) in both Zebra mussels and Asian clams, and found that the Asian clams were 2-7 time more tolerant of hypoxia than zebra mussels exposed to same conditions at the same temperatures. Asian clams could survive a mean of 11.8 days to extreme hypoxia at 25°C, 35.1 days at 15°C, and without mortality at 84 days at 5°C (Matthews and McMahon 1999). Valve closure and an ability to reduce metabolism helps to save energy and may be a factor of why Asian clams appear to remain unaffected by elevated pH for such a long period of time compared to other species tested. These studies suggest the Asian clam is able to withstand harsh conditions.

The reduced metabolic rate rather than valve closure may be the reason that time to mortality was higher for some of our test species. Bidwell et al. (1995) used small pieces of toothpick glued between the valves of the Asian clams being exposed to a molluscicide (DGH/QUAT)

so they could not close their valves and avoid exposure. They found no significant difference in mortality between the clams that were pegged open with toothpicks and unpegged ones, indicating that unpegged clams did not avoid the molluscicide by valve closure (Bidwell et al. 1995). In my experiments, mollusks were observed to be tightly closed and sealed inside their shells prior to exposure to test solution. The study by Bidwell et al. (1995) suggests that valve closure has no effect on time to mortality when Asian clams are exposed to a molluscicide. Perhaps the low mortality rate observed in Asian clams is more a factor of its ability to reduce its metabolic rate than its ability to close. This study also suggests that using a pre-treatment chemical to relax the mollusks prior to exposure to test solution, to reduce time to mortality, may not be necessary if valve closure is not an issue in preventing exposure of toxins to sensitive parts.

My results support my hypothesis that warm temperature exposures reduce the time to mortality. This same trend can be found in other studies. Matthews and McMahon (1999) who examined the effects of different temperatures on tolerance to extreme hypoxia in zebra mussels and Asian clams found that hypoxia tolerance increased significantly with decreasing temperature in both Asian clams and zebra mussels. Johnson and McMahon (1998) also found increased survivor ship of Asian clams and zebra mussels at decreased temperatures in their studies with chronic hypoxia. Mollusks are poikilotherms and their metabolic rate (which can be measured by heart rate) is affected by environmental temperatures (Pickens 1965; Polhill and Dimock 1996; Braby and Somero 2006). Increased heart rate at upper temperature tolerances is a sign of thermal stress (Pandolfo et al. 2009). Although heart rate was not monitored in my experiments, individuals tested at warmer test temperatures with elevated pH

were likely under more thermal stress than those conducted at cooler test temperatures. Increased heart rate increases the rate of respiration, which consumes available oxygen and this lack of oxygen may have caused our mollusks to open earlier than those at colder test temperature, which resulted in a more rapid time to mortality for tests in warmer experiment.

My experiments supported the hypothesis that life stage and developmental form within life stage affect time to mortality. In our experiments the veliger and neonate life stages died at a much faster rate in elevated pH solutions than the adult life stages in all species tested. Veligers develop very rapidly and increased temperatures can help speed this process, however there are variances in the time required to reach successional forms and researchers have found that different veliger forms can grow at different rates in the same environmental conditions (Sprung 1993, Nicholes 1996). For example, in zebra mussels, veligers can develop from a fertilized egg to a pediveliger (the final larval form) in anywhere from 18 to 90 days (Environmental Laboratory 2015). Increased shell development through the different successional forms as veligers could have helped to lower sensitivity in tests with veligers as the days progressed. Harrison et al. (1984) found that sensitivity to copper exposure decreased with successional life stages of Asian clams, with juveniles being less sensitive to copper than veligers. They also found that increased shell development in veligers also reduced sensitivity with trochophore larvae being more sensitive than shell developed veligers (Harrison et al. 1984). Edwards et al. (2000) found a similar response when different developmental stages of zebra mussel veligers were exposed to potassium chloride. Edward et al. (2000) found that post-D veligers were less sensitive then preshell veligers. In my experiments different forms of veligers were not separated. Shell development and size variation was likely the reason we

saw a reduction in mortality response in the experiment with Asian clam veligers in the 11.8 pH treatment compared to those tested at 11.4 and 12 pH. Veligers in the test conducted at 11.8 pH were believed to be 1 week older than veligers used in the other tests at 11.4 and 12 pH. Had all tests been conducted within the same week with veligers that were in the same developmental form veligers in the 11.8 pH may have experienced an earlier time to mortality than veligers in the 11.4 pH. However tests were conducted a couple weeks apart and according to the cited literature a variety of veliger forms was likely tested as the week progressed. Only a limited number of veligers in the trials were measured and so size and shell development could have influenced time to mortality.

Based on my experiments, I concluded that quagga mussels were very sensitive to brackish water conditions. Juvenile mussel mortality in NaOH with brackish water occurred 6 times faster than in freshwater. This same trend was also seen with adult quagga mussels, with mortality for adults tested in brackish water occurring over 2 times faster than adults tested in fresh water. Spidle et al. (1995) who examined the tolerance of temperature and salinity of quagga and zebra mussels found that neither species tolerated salinities higher than 5 ppt for over 18 days. Many ships are required to flush their tanks with ocean water before entering port and while an open ocean exchange may prevent a majority of invasive species from surviving, not all species are affected by oceanic salinities. For example, NZMS are active in salinities as high as 17 ppt (Winterbourn 1970) and in my studies NZMS were not affected by brackish water conditions of 15 ppt. In trials conducted at Willow Beach Arizona, which has both NZMS and quagga mussels, there were instances during which the species were mixed in test containers and NZMS actively crawled in 15 ppt sea water, whereas quaggas were tightly

closed and immobile. Asian clams can tolerate salinities of 24 ppt (Evans et al. 1979). Due to time limitations, no brackish water tests were conducted with NZMS or Asian calms, but based on their tolerance to salinity it is expected that 15 ppt brackish would have little to no effect on increasing mortality when combined with elevated pH. The differences in environmental tolerances of these species highlights why additional measures such as the presence of elevated pH is necessary to create undesirable conditions for species hardest to kill. An open ocean exchange alone may not provide a sufficiently harsh environment to eradicate all potential species in the ballast.

Study Limitations

Tight 95% confidence intervals on either side of the mean lethal time to mortality suggest a more precise estimate of the lethal time to mortality whereas wide confidence intervals are less precise. Confidence intervals become tighter (more precise) when the number of trials, replicates, and test individuals per replicate is high. Some tests in this study were limited in their scope (low number of test individuals or replicates within sampling intervals) and therefore the confidence intervals are wider. Additional trials should be conducted for those trials with low number of test organisms or sampling intervals to improve confidence of the precise time to mortality. Also, when the lethal time to 99% was estimated by probit regression there was no subtraction of control mortalities from the data. As a result any mortality (natural or by handling) that occurred would cause the model to estimate a more rapid time to 99% mortality. Regulations by EPA were used for determining if these trials were within acceptable limits.

Only a limited number of trials were conducted to explore the efficacy of brackish solutions at 15 ppt. Asian clams and New Zealand mudsnails were not tested. Both these species appear tolerant of brackish challenges (Winterbourn 1970; Evans et al 1979), but we do not know the synergy of pH with elevated salinity. In some cases certain systems may not be able to reach or tolerate a pH 12 treatment. Because of this, additional tests with lower pH dosages should be undertaken. If a lower pH is found to be lethal to invasive mollusks, less chemical product would need to be used which would be more economical.

Studies have found that potassium salts can cause invertebrates such as Asian calms to become relaxed (Fisher et al. 1991). Potassium used with elevated pH may further help reduce time to mortality by relaxing invertebrates, causing them to open and siphon the toxic solutions. The amount of potassium salts needed in combination with elevated pH chemicals to achieve relaxation is not known but should be investigated. Edwards et al. (2000) found that potassium chloride (KCl) alone was ineffective at killing veliger zebra mussels, even at very high concentrations (> 6000 mg/L for 3 h). However, when combined in lower doses as a pretreatment (1 h exposure) with other chemicals such as formalin, Edwards et al. (2000) found it killed 100% of veligers after a 2 h exposure and was also safe for several different fish species. The authors did find however, that when KCl was combined with sodium chloride (NaCl) the effect of KCl was reduced or had no effect. If effectiveness of elevated pH could be increased and the time to mortality reduced with a pretreatment that had similar effects on mussels as KCl it may make for a more economically friendly tool, but this needs to be further investigated. Future studies should investigate different application techniques. For example, if an elevated pH solution was applied at a slower rate (flow through system

design) it may have time to be ingested by the mollusks rather than applying solution to already closed individuals. Future studies should also look into the effects of direct exposures of invasive mollusks to elevated pH by injection of the solution into the mantel cavities to determine if direct contact results in a more rapid time to mortality. If this is the case then a pre-treatment option may be worthwhile in cases where adult life stages are present.

Elevated pH may have the potential to be used as a rapid response tool. In Lake Tahoe, located on the Arizona California border, invasive Asian clam populations have been managed by lining the benthos with impermeable barriers which contain Aspen fiber (Wittmann et al. 2012). As this fiber degrades under the mats it consumes oxygen and creates anoxic conditions for the clams ultimately suffocating them. NaOH pellets may be able to be used to create a toxic barrier under mats to help induce mortality, reduce the time to mortality and also reduce the time of barrier deployment.

Conclusions/Management Implications

Based on the results and observations of this study it is recommend to use NaOH over $Ca(OH)_2$ for applications in ship ballast tanks. Although $Ca(OH)_2$ is probably a less expensive option, this chemical does not hydrate as readily as NaOH and results in more of a slurry than a well-mixed solution. This slurry may cause issues within certain structures such as pipes and cause calcification. If not properly flushed out this residue can build up and potentially seal off pipes or scale the inside of ballast tanks. Warmer water conditions or heating ballast tanks where possible is recommended as this helps to decrease the time to mortality and increase treatment efficacy than when colder water is used. Also, brackish water of 15 ppt or above

can help increase mortality depending on the target species. Life stage is also an important consideration. If no adult stages of these target mollusk species are present, disinfection treatments of 1.5 h at 20°C may be fully effective. If adult life stages are present then more than 10 days may be needed to achieve complete mortality of Asian clams. The presence of adults in ballast systems is rare and so disinfection times of several hours or weeks is unlikely.

Caution should be taken when deciding on an effective exposure time as the robustness of species can vary seasonally and geographically due to various environmental conditions. For the New Zealand mudsnail and the Asian clam that have clonal or hermaphroditic reproduction, only a single mature organism needs to survive to begin a new population. To ensure complete mortality the upper 95% confidence interval of the probit model estimate is likely a more appropriate target for management (Table 36 and 37).

References

Aldridge, D. W., and R. F. McMahon. 1978. Growth, fecundity, and bioenergetics in a natural population of the Asiatic freshwater clam, *Corbicula manilensis* Philippi, from North Central Texas. Journal of Molluscan Studies 44:49-70.

Balcom, N. 1994. Aquatic immigrants of the Northeast, No. 4: Asian clam, *Corbicula fluminea*. University of Connecticut. Connecticut Sea Grant College Program, Reference number 13203, Groton.

Benson, A.J., M. M. Richerson, E. Maynard, J. Larson, A. Fusaro, and A. K. Bogdanoff. 2014. *Dreissena rostriformis bugensis*. USGS Nonindigenous Aquatic Species Database, Gainesville, FL. http://nas.er.usgs.gov/queries/factsheet.aspx?speciesid=95 Revision Date: 6/26/2014.

Bidwell, J. R., J. L. Farris, and D. S. Cherry. 1995. Comparative response of the zebra mussel, *Dreissena polymorpha*, and the Asian clam, *Corbicula fluminea*, to DGH/QUAT, a nonoxidizing molluscicide. Aquatic toxicology 33:183-200.

Boltovskoy, D., I. Izaguirre, and N. Correa. 1995. Feeding selectivity of *Corbicula fluminea* (Bivalvia) on natural phytoplankton. Hydrobiologia 312:171-182.

Bondesen, P., and E. Kaiser. 1949. *Hydrobia* (Potamopyrgus) *jenkinsi* Smith in Denmark illustrated by its ecology. Oikos 1:252-281.

Bowler, P. A. 1991. The rapid spread of the freshwater hydrobiid snail *Potamopyrgus antipodarum* (Gray) in the Middle Snake River, southern Idaho. Pages 173-182 *in* Proceedings of the Desert Fishes Council.

Bowler, P., and T. Frest. 1996. The advancing distribution of the New Zealand mud snail, *Potamopyrgus antipodarum* (Gray). North America. American Malacological Union 62nd Annual Meeting Program and Abstracts.

Braby, C. E., and G. N. Somero. 2006. Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus Mytilus). Journal of Experimental Biology 209:2554-2566.

Bruce, R. L., C. M. Moffitt, and B. Dennis. 2009. Survival and passage of ingested New Zealand mudsnails through the intestinal tract of rainbow trout. North American Journal of Aquaculture 71:287-301.

Bruce, R. L., and C. M. Moffitt. 2010. Quantifying risks of volitional consumption of New Zealand mudsnails by steelhead and rainbow trout. Aquaculture Research 41:552-558.

Cangelosi A., L. Allinger, M. Balcer, L. Fanberg, S. Hagedorn, T. Markee, N. Mays, C. Polkinghorne, K. Prihoda, E. Reavie, D. Regan, D. Reid, E. Ruzycki, H. Saillard, T. Schwerdt, H. Schaefer, and M. TenEyck. 2011. Final report of the land-based freshwater testing of the lye (NaOH) ballast water treatment system. Great Ships Initiative, Northeast-Midwest Institute, Washington, D.C., USA.

Cangelosi A., L. Allinger, M. Balcer, L. Fanberg, D. Fobbe, S. Hagedorn, T. Mangan, A. Marksteiner, N. Mays, C. Polkinghorne, K. Prihoda, E. Reavie, D. Regan, D. Reid, E. Ruzycki, H. Saillard, H. Schaefer, T. Schwerdt, and M. TenEyck. 2013. Final report of the shipboard testing of the sodium hydroxide (NaOH) ballast water treatment system onboard the MV Indiana Harbor. Great Ships Initiative, Northeast-Midwest Institute, Washington, D.C., USA.

Chan, F. T., S. A. Bailey, H. J. MacIsaac. Arctic shipping: a free ride for aquatic invasive species. 2013, January 3. Meridian newsletter: fall/winter 2011-spring/summer 2012 – artic shipping. Retrieved from http://www.polarcom.gc.ca/eng/content/meridian-newsletter-fallwinter-2011-springsummer-2012-1.

Cheng, Y. W., and L. L. LeClair. 2011. A quantitative evaluation of the effect of freezing temperatures on the survival of New Zealand mudsnails (*Potamopyrgus antipodarum* Gray, 1843). Aquatic Invasions 6:47-54.

Claudi, R., and G. L. Mackie. 1993. Practical manual for the monitoring and control of macrofouling mollusks in fresh water sys. Chapter 3. Zebra Mussel Infestation: Structures of Concern CRC Press.

Claudi, R., A. Graves, A. C. Taraborelli, R. J. Prescott, and S. E. Mastitsky. 2012. Impact of pH on survival and settlement of *Dreissenid* mussels. Aquatic Invasions 7:21-28.

ClearTech Industries Inc.. 2009. MSDS calcium hydroxide. Pages 1-7 *in* Chemical and Product and Company Information, Saskatoon, SK. Canada.

Connelly, N. A., C. R. O'Neill Jr, B. A. Knuth, and T. L. Brown. 2007. Economic impacts of zebra mussels on drinking water treatment and electric power generation facilities. Environmental Management 40:105-112.

Corn, L.C., E.H. Buck, J. Rawson, and E. Fischer. 1999. Harmful Non-Native Species: Issues for Congress. Congressional Research Service Issue Brief, RL30123.

Counts, C. L. 1981. *Corbicula fluminea* (Bivalvia: Sphaeriacea) in British Columbia. Nautilus 95:12-13

Cox, T. J., and J. C. Rutherford. 2000. Thermal tolerances of two stream invertebrates exposed to diumally varying temperature. New Zealand Journal of Marine and Freshwater Research 34:203-208.

Coyne, K. J., X.-X. Qin, and J. H. Waite. 1997. Extensible collagen in mussel byssus: a natural block copolymer. Science 277:1830-1832.

DeBruyckere L. A., and S. H. Phillips. 2014. Strategies to conduct vulnerability assessments for high priority Columbia River Basin hydropower and dam Facilities. Pacific States Marine Fisheries Commission.

Dermott, R., and M. Munawar. 1993. Invasion of Lake Erie offshore sediments by *Dreissena*, and its ecological implications. Canadian Journal of Fisheries and Aquatic Sciences 50:2298-2304.

Echols, R. 2013, August 6. Rosalind Echols: cool science on the ship and final reflections on my rainier adventure, July 30, 2013. NOAA teacher at sea. Retrieved from https://teacheratsea.wordpress.com/category/rosalind-echols/.

Edwards, W. J., L. Babcock-Jackson, and D. A. Culver. 2000. Prevention of the spread of zebra mussels during fish hatchery and aquaculture activities. North American Journal of Aquaculture 62:229-236.

Evans Jr, L. P., C. E. Murphy, J. C. Britton, and L. W. Newland. 1979. Salinity relationships in *Corbicula fluminea* (Müller). Pages 193-214 *in* Proceedings of the First International Corbicula Symposium.

Fisher, S. W., P. Stromberg, K. A. Bruner, and L. D. Boulet. 1991. Molluscicidal activity of potassium to the zebra mussel, *Dreissena polymorphia*: toxicity and mode of action. Aquatic Toxicology 20:219-234.

Frisch, D., A. J. Green, and J. Figuerola. 2007. High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. Aquatic Sciences 69:568-574.

Foster, A.M., P. Fuller, A. Benson, S. Constant, D. Raikow, J. Larson, and A. Fusaro. 2014. *Corbicula fluminea*. USGS Nonindigenous Aquatic Species Database, Gainesville, FL.
http://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=92 Revision Date: 6/26/2014.

Gutiérrez, J. L., C. G. Jones, D. L. Strayer, and O. O. Iribarne. 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. Oikos 101:79-90.

Hakenkamp, C. C., and M. A. Palmer. 1999. Introduced bivalves in freshwater ecosystems: the impact of *Corbicula* on organic matter dynamics in a sandy stream. Oecologia 119:445-451.

Hakenkamp, C. C., S. G. Ribblett, M. A. Palmer, C. M. Swan, J. W. Reid, M. R. Goodison. 2001. The impact of an introduced bivalve (*Corbicula fluminea*) on the benthos of a sandy stream. Freshwater Biology 46:491-501.

Hall Jr, R. O., J. L. Tank, and M. F. Dybdahl. 2003. Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. Frontiers in Ecology and the Environment 1:407-411.

Hall Jr, R. O., M. F. Dybdahl, and M. C. VanderLoop. 2006. Extremely high secondary production of introduced snails in rivers. Ecological Applications 16:1121-1131.

Harrison, F. L., J. P. Knezovich, and D. W. Rice Jr. 1984. The toxicity of copper to the adult and early life stages of the freshwater clam, *Corbicula manilensis*. Archives of Environmental Contamination and Toxicology 13:85-92.

Haynes, A., B. Taylor, and M. Varley. 1985. The influence of the mobility of *Potamopyrgus jenkinsi* (Smith, EA) (Prosobranchia: Hydrobiidae) on its spread. Archiv für Hydrobiologie 103:497-508.

Hinderer, J. M., M. W. Murray, and T. Becker. 2011. Feast and famine in the Great Lakes: how nutrients and invasive species interact to overwhelm the coasts and starve offshore waters. National Wildlife Federation, http://www.nwf.org/~/media/PDFs/Regional/Great-Lakes/GreatLakes-Feast-and-Famine-Nutrient-Report.ashx

International Maritime Organization (IMO). 2015. Ballast water management. Retrieved January 16, 2015 from http://www.imo.org/OurWork/Environment/BallastWaterManagement/Pages/Default.aspx

Independent Economic Analysis Board (IEAB). 2013. Economic risk of zebra mussels and quagga mussels in the Columbia River Basin.

Johnson, P. D., and R. F. McMahon. 1998. Effects of temperature and chronic hypoxia on survivorship of the zebra mussel (*Dreissena polymorpha*) and Asian clam *(Corbicula fluminea*). Canadian Journal of Fisheries and Aquatic Sciences 55:1564-1572

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

Jones, L. A., and A. Ricciardi. 2005. Influence of physicochemical factors on the distribution and biomass of invasive mussels *(Dreissena polymorpha* and *Dreissena bugensis*) in the St. Lawrence River. Canadian Journal of Fisheries and Aquatic Sciences 62:1953-1962.

Karatayev, A. Y., L. E. Burlakova, T. Kesterson, and D. K. Padilla. 2003. Dominance of the Asiatic clam, *Corbicula fluminea* (Muller), in the benthic community of a reservoir. Journal of Shellfish Research 22:487-494.

Karatayev, A. Y., D. K. Padilla, D. Minchin, D. Boltovskoy, and L. E. Burlakova. 2007. Changes in global economies and trade: the potential spread of exotic freshwater bivalves. Biological Invasions 9:161-180.

Keller, R. P., J. M. Drake, and D. M. Lodge. 2007. Fecundity as a basis for risk assessment of nonindigenous freshwater molluscs. Conservation Biology 21:191-200.

Keller, R. P., K. Frang, and D. M. Lodge. 2008. Preventing the spread of invasive species: economic benefits of intervention guided by ecological predictions. Conservation Biology 22:80-88.

Kerans, B., M. Dybdahl, M. Gangloff, and J. Jannot. 2005. *Potamopyrgus antipodarum*: distribution, density, and effects on native macroinvertebrate assemblages in the Greater Yellowstone Ecosystem. Journal of the North American Benthological Society 24:123-138.

Kraemer, L.R., C. Swanson, M. Galloway, and R. Kraemer. 1986. Biological basis of behavior in *orbicula fluminea*. II. Functional morphology of reproduction and development and review of evidence for self-fertilization. American Malacological Bulletin. Special Edition 2: 193–201.

Krist, A. C., and C. M. Lively. 1998. Experimental exposure of juvenile snails (*Potamopyrgus antipodarum*) to infection by trematode larvae (*Microphallus sp*.): infectivity, fecundity compensation and growth. Oecologia 116:575-582.

Lassen, H. H. 1979. Reproductive effort in Danish mudsnails (*Hydrobiidae*). Oecologia 40:365-369.

Lauritsen, D. D. 1986. Filter-feeding in *Corbicula fluminea* and its effect on seston removal. Journal of the North American Benthological Society: 165-172.

Leung, B., D. M. Lodge, D. Finnoff, J. F. Shogren, M. A. Lewis, and G. Lamberti. 2002. An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. Proceedings of the Royal Society of London. Series B: Biological Sciences 269:2407-2413.

Lewandowski, K., 1976: Unionidae as a substratum for Dreissena polymorpha PALLAS. Polskie Archiwum Hydrobiologii. 23:409–420.

Mackie, G. L., and R. Claudi. 2009. Monitoring and control of macrofouling mollusks in fresh water systems. Chapter 1. Biology. CRC Press Inc.

Markestad, J. K. 2010. Sodium hydroxide (NaOH) practicality study. The Glosten Associates, 09129.01, Seattle, Washington.

Matthews, M. A., and R. F. McMahon. 1999. Effects of temperature and temperature acclimation on survival of zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) under extreme hypoxia. Journal of Molluscan Studies 65:317-325.

McArthur, C. P., and D. Featherston. 1976. Suppression of egg production in *Potamopyrgus antipodarum* (Gastropoda: Hydrobiidae) by larval trematodes. New Zealand Journal of Zoology 3:35-38.

McCarter, N. H. 1986. Food and energy in the diet of brown and rainbow trout from Lake Benmore, New Zealand. New Zealand Journal of Marine and Freshwater Research 20:551-559.

McMahon, R. F. 1982. The Occurrence and spread of the introduced Asiatic fresh water clam, *Corbicula-fluminea* (Muller), in North America 1924-1982. Nautilus 96:134-141.

McMahon, R. F. 1991. Mollusca: bivalvia. Pp. 315-390 in Thorp J. H. & Covich A. P., editors. Ecology and Classification of North American Freshwater Invertebrates. Academic Press, New York.

McMahon, R. F. 2000. Invasive characteristics of the freshwater bivalve Corbicula fluminea. In R. Claudi & J. Leach, editors. Nonindigenous Freshwater Organisms: Vectors, Biology, and Impacts. Lewis Publishers, Boca Raton, FL, pp 315-343.

McMahon, R. F. 2002. Evolutionary and physiological adaptations of aquatic invasive animals: r selection versus resistance. Canadian Journal of Fisheries and Aquatic Sciences 59:1235-1244.

Mills, E. L., G. Rosenberg, A. P. Spidle, M. Ludyanskiy, Y. Pligin, and B. May. 1996. A review of the biology and ecology of the quagga mussel (*Dreissena bugensis*), a second species of freshwater dreissenid introduced to North America. American Zoologist 36:271-286.

Moffitt, C. M., A. Barenberg, K. A. Stockton, and B. J. Watten. In press. Efficacy of two approaches for disinfecting surfaces and water infested with quagga mussel veligers. Pp XX in W. H. Wong and S. Gerstenberger, editors. Biology and management of invasive quagga and zebra mussels in the Western United States. CRC press.

Moffitt, C. M., and C. A. James. 2012. Dynamics of *Potamopyrgus antipodarum* infestations and seasonal water temperatures in a heavily used recreational watershed in intermountain North America. Aquatic Invasions 7:193-202.

Nichols, S. J. 1996. Variations in the reproductive cycle of Dreissena polymorpha in Europe, Russia, and North America. American Zoologist 36(3):311-325.

Nielson, R. J., C. M. Moffitt, and B. J. Watten. 2012a. Hydrocyclonic separation of invasive New Zealand mudsnails from an aquaculture water source. Aquaculture 326:156-162.

Nielson, R. J., C. M. Moffitt, and B. J. Watten. 2012b. Toxicity of elevated partial pressures of carbon dioxide to invasive New Zealand mudsnails. Environmental Toxicology and Chemistry 31:1838-1842.

OECD SIDS. 2002. Sodium hydroxide. Screening Information Data Set. Organization for Economic Cooperation and Development. , Paris.

Oregon Sea Grant. 2010. Zebra and Quagga Mussels. Oregon State University.

Ortmann, C., and M. K. Grieshaber. 2003. Energy metabolism and valve closure behaviour in the Asian clam *Corbicula fluminea*. Journal of Experimental Biology 206:4167-4178.

Pace, M. L., S. E. Findlay, and D. Fischer. 1998. Effects of an invasive bivalve on the zooplankton community of the Hudson River. Freshwater Biology 39:103-116.

Pandolfo, T. J., W. G. Cope, and C. Arellano. 2009. Heart rate as a sublethal indicator of thermal stress in juvenile freshwater mussels. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 154(3):347-352.

Phelps, H. L. 1994. The Asiatic clam (*Corbicula fluminea*) invasion and system-level ecological change in the Potomac River estuary near Washington, DC. Estuaries 17:614-621.

Pickens, P. E. 1965. Heart rate of mussels as a function of latitude, intertidal height, and acclimation temperature. Physiological Zoology:390-405.

Pigneur, L. M., E. Falisse, K. Roland, E. Everbecq, J. F. Deliege, J. S. Smitz, K. Doninck, and J. P. Descy. 2014. Impact of invasive Asian clams, *Corbicula spp*., on a large river ecosystem. Freshwater Biology 59:573-583.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. Bioscience, 50:53-65.

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

Polhill V, J. B., and R. V. Dimock Jr. 1996. Effects of temperature and pO2 on the heart rate of juvenile and adult freshwater mussels (Bivalvia: Unionidae). Comparative Biochemistry and Physiology Part A: Physiology 114(2):135-141.

Ram, J. L., P. P. Fong, and D. W. Garton. 1996. Physiological aspects of zebra mussel reproduction: Maturation, spawning, and fertilization. American Zoologist 36:326-338.

Ricciardi, A., R. J. Neves, and J. B. Rasmussen. 1998. Impending extinctions of North American freshwater mussels (*Unionoida*) following the zebra mussel (*Dreissena polymorpha*) invasion. Journal of Animal Ecology 67:613-619.

Ricciardi, A., and H. J. MacIsaac. 2000. Recent mass invasion of the North American Great Lakes by Ponto–Caspian species. Trends in Ecology & Evolution 15:62-65.

Richards, D. C., P. O'Connell, and D. C. Shinn. 2004. Simple control method to limit the spread of the New Zealand mudsnail *Potamopyrgus antipodarum*. North American Journal of Fisheries Management 24:114-117.

Roe, S. L., and H. J. MacIsaac. 1997. Deepwater population structure and reproductive state of quagga mussels (*Dreissena bugensis*) in Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 54:2428-2433.

Rothlisberger, J. D., W. L. Chadderton, J. McNulty, and D. M. Lodge. 2010. Aquatic invasive species transport via trailered boats: what is being moved, who is moving it, and what can be done. Fisheries 35:121-132.

Scheller, J. L. 1997. The effect of dieoffs of Asian clams (*Corbicula fluminea*) on native freshwater mussels (*Unionidae*). Virginia Polytechnic Institute and State University.

Schloesser, D. W., T. F. Nalepa, and G. L. Mackie. 1996. Zebra mussel infestation of unionid bivalves (*Unionidae*) in North America. American Zoologist 36:300-310.

Semmens, B. X., E. R. Buhle, A. K. Salomon, and C. V. Pattengill-Semmens. 2004. A hotspot of non-native marine fishes: evidence for the aquarium trade as an invasion pathway. Marine Ecology Progress Series 266:239-244.

Silverman, H., E. Achberger, J. Lynn, and T. Dietz. 1995. Filtration and utilization of laboratory-cultured bacteria by *Dreissena polymorpha, Corbicula fluminea*, and *Carunculina texasensis*. The Biological Bulletin 189:308-319.

Solvay Sustainable. 2011. Product safety summary of calcium carbonate. Brussels, Belgium.

Solvay Sustainable. 2012. Product safety summary of sodium carbonate (soda ash). Brussels, Belgium.

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

Sprung, M. 1993. The other life: an account of present knowledge of the larval phase of *Dreissena polymorpha.* Pp. 39-53 In T. F. Nalepa and D. Schloesser, editors. Zebra mussels: Biology, impacts, and control. Boca Raton, Fla. CRC Press.

Starliper, C. E., and B. J. Watten. 2013. Bactericidal efficacy of elevated pH on fish pathogenic and environmental bacteria. Journal of Advanced Research 4:345-353.

Stockton, K. A., and C. M. Moffitt. 2013. Disinfection of three wading boot surfaces infested with New Zealand Mudsnails. North American Journal of Fisheries Management 33:529-538.

Strayer, D. L., K. A. Hattala, and A. W. Kahnle. 2004. Effects of an invasive bivalve (*Dreissena polymorpha*) on fish in the Hudson River estuary. Canadian Journal of Fisheries and Aquatic Sciences 61:924-941.

Strzelec, M. 2005. Impact of the introduced *Potamopyrgus antipodarum* (Gastropoda) on the snail fauna in post-industrial ponds in Poland. Biologia 60:159-163.

TenEyck, M., and A. Cangelosi. 2009. Bench-scale test findings: sodium hydroxide (NaOH). Great Ships Initiative, Northeast-Midwest Institute, Washington, D. C., USA.

TenEyck, M., N. Mays, and A. Cangelosi 2011. Bench-scale test findings: hydrated lime, $Ca(OH)_2$. Great Ships Initiative, Northeast-Midwest Institute, Washington, D. C., USA.

Tsolaki, E., and E. Diamadopoulos. 2010. Technologies for ballast water treatment: a review. Journal of Chemical Technology and Biotechnology 85:19-32.

Turner, K., W. H. Wong, S. Gerstenberger, and J. M. Miller. 2011. Interagency monitoring action plan (I-MAP) for quagga mussels in Lake Mead, Nevada-Arizona, USA. Aquatic Invasions 6:195.

United Nations Environment Programme (UNEP). n.d. Invasive alien species – a growing threat in regional seas. Regional Seas Programme. Brochure. Modified February 10 2015. http://www.unep.org/regionalseas/publications/brochures/pdfs/invasive_alien_brochure.pdf

U.S. Department of Energy. 1993. DOE fundamentals handbook chemistry. Pages 11-20 *in* U. S. D. O. Energy, editor. U.S. Department of Energy, Washington, D.C.

U.S. Department of Fish and Wildlife Service. 2004. Risk assessment and risk management recommendations for New Zealand Mudsnail introduction from Hagerman NFH Steelhead releases. Idaho Fishery Resource Office, Ahsahka, Idaho.

U.S. Fish and Wildlife Service, Invasive Species. 2012. Frequently asked question about invasive species. Retrieved from http://www.fws.gov/invasives/faq.html#q7

U.S. Department of Health and Human Services. 2002. Sodium hydroxide. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services.

United States Environmental Protection Agency (USEPA). 2001. Final Report: Interlaboratory Variability Study of EPA Short-Term Chronic and Acute Whole Effluent Toxicity Test Methods, Vol. 1. United States Environmental Protection Agency Office of Water, Washington, D.C.. EPA/821/B-01/004.

United States Environmental Protection Agency (USEPA). 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA/821/R-02/012. 9, 50 pp.

U.S. Environmental Protection Agency. 2006. Reregistration eligibility decision for sodium carbonate; Weak Mineral Bases. Pages vi. Office of Prevention, Pesticides and Toxic Substances, Washington, D.C.

U.S Federal Register. 2014. Ballast water management for control of nonindigenous species in the Great Lakes and Hudson River. 33 CFR Part 151.1510: Navigation and Navigable Water.

Vanschoenwinkel, B., A. Waterkeyn, T. Vandecaetsbeek, O. Pineau, P. Grillas, and L. Brendonck. 2008. Dispersal of freshwater invertebrates by large terrestrial mammals: a case study with wild boar (*Sus scrofa*) in Mediterranean wetlands. Freshwater Biology 53:2264-2273.

Vinson, M. R., and M. A. Baker. 2008. Poor growth of rainbow trout fed New Zealand mud snails *Potamopyrgus antipodarum*. North American Journal of Fisheries Management 28:701-709.

Wakefield, K. and A. Faulds, editors. 2003. Economic and ecological costs associated with Aquatic Invasive Species. Proceedings of the Aquatic Invaders of the Delaware Estuary Symposium, Malvern, Pennsylvania, May 20, 2003. Pp. 3-5

Warziniack T. W., D. Finnoff, J. F. Shogren. 2013. Public economics of hitchhiking species and tourism-based risk to ecosystem services. Resource and Energy 35:277-294.

Waterkeyn, A., B. Vanschoenwinkel, S. Elsen, M. Anton-Pardo, P. Grillas, and L. Brendonck. 2010. Unintentional dispersal of aquatic invertebrates via footwear and motor vehicles in a Mediterranean wetland area. Aquatic Conservation: Marine and Freshwater Ecosystems 20:580-587.

Way, C. M., D. J. Hornbach, C. A. Miller-Way, B. S. Payne, and A. C. Miller. 1990. Dynamics of filter feeding in *Corbicula fluminea* (Bivalvia: Corbiculidae). Canadian Journal of Zoology 68:115-120.

Western Chemical. 2013. Virkon Aquatic - Virkon Aquatic - Biosecurity Supplies / Disinfectants - Products. W. Chemical, editor, Ferndale, WA 98248 USA.

Whittier, T. R., P. L. Ringold, A. T. Herlihy, and S. M. Pierson. 2008. A calcium-based invasion risk assessment for zebra and quagga mussels (*Dreissena spp*). Frontiers in Ecology and the Environment 6:180-184.

Williams, R. J., F. B. Griffiths, E. J. Van der Wal, and J. Kelly. 1988. Cargo vessel ballast water as a vector for the transport of non-indigenous marine species. Estuarine, Coastal and Shelf Science 26:409-420.

Winterbourn, M. 1969. Water temperature as a factor limiting the distribution *of Potamopyrgus antipodum* (Gastropoda-Prosobranchia) in the New Zealand thermal region. New Zealand Journal of Marine and Freshwater Research 3:453-458.

Winterbourn, M. 1970. Population studies on the New Zealand freshwater gastropod, *Potamopyrgus Antipodarum* (Gray). Journal of Molluscan Studies 39:139-149.

Wittmann, M. E., S. Chandra, J. Reuter, S. G. Schladow, B. C. Allen, and K. J. Webb. 2012. The control of an invasive bivalve, *Corbicula fluminea*, using gas impermeable benthic barriers in a large natural lake. Environmental Management 49:1163-1173.

Yan, X., L. Zhenyu, W. P. Gregg, and L. Dianmo. 2001. Invasive species in China—an overview. Biodiversity & Conservation 10:1317-1341.

Yeager, M. M., R. J. Neves, and D. S. Cherry. 2000. Competitive interactions between early life stages of *Villosa iris* (Bivalvia: Unionidae) and adult Asian clams (*Corbicula fluminea*). Pages 253-259 in P. D. Johnson and R. S. Butler, editors. Freshwater Mollusk Symposium Proceedings –Part II: Proceedings of the First Freshwater Mollusk Conservation Society Symposium, March 1999. Ohio Biological Survey, Columbus, Ohio.

Zaranko, D. T., D. G. Farara, and F. G. Thompson. 1997. Another exotic mollusc in the laurentian great lakes: the New Zealand native *Potamopyrgus antipodarum* (Gray 1843)(Gastropoda, Hydrobiidae). Canadian Journal of Fisheries and Aquatic Sciences 54:809-814.

Table 1. Summary of all toxicology trials and the environmental parameters tested. There were a total of 28 trials conducted for this study. Test replicates were either tea balls, glass beakers, or plastic containers that held several organisms. Number of test replicates for each time interval in the experiment is listed.

Species	Lifestage	Test water	Chemical	Target ph	Test temp range °C	Number of trials	Number of test replicates
NZMS	adult	fresh	CaOH	12.0	18-20	1	5
NZMS	adult	fresh	NaOH	12.0	22-23	1	2
NZMS	adult	fresh	NaOH	11.8	21-22	1	2
NZMS	Neonate	fresh	NaOH	12.0	19-21	4	2 - 3
Asian Clam	adult	fresh	CaOH	11.8	22-26	1	2
Asian Clam	adult	fresh	NaOH	12.0	20-22	2	4
Asian Clam	veliger	fresh	CaOH	12.0	21-22	1	3
Asian Clam	veliger	fresh	CaOH	11.8	21-22	1	3
Asian Clam	veliger	fresh	CaOH	11.4	21-23	2	3 - 4
Asian Clam	veliger	fresh	CaOH	11.2	24-25	1	3
Quagga	adult	fresh	CaOH	12.0	18-20	1	3
Quagga	adult	fresh	NaOH	12.0	18-21	2	2 - 3
Quagga	adult	fresh	NaOH	12.0	16-17	1	3
Quagga	adult	brackish	NaOH	12.0	20-22	2	3
Quagga	Juvenile	fresh	NaOH	12.0	20-22	1	4
Quagga	Juvenile	brackish	NaOH	12.0	20-21	2	4
Quagga	veliger	fresh	NaOH	12.0	20	1	4
Quagga	veliger	fresh	NaOH	12.0	15	1	4
Quagga	veliger	fresh	CaOH	12.0	20	1	4
Quagga	veliger	fresh	CaOH	12.0	15	1	4
Total						28	

Table 2. Summary of pH, temperature, conductivity, and dissolved oxygen in test and control solution of trials with New Zealand mudsnails. Target solutions of pH 12 were prepared with either $Ca(OH)_2$ abbreviated C or NaOH abbreviated N. Controls were de-chlorinated well water. Tests and controls are separated by life stage (adult or neonate). Tests and recovery were in fresh water.

Trial ID	Test date start	Chemical	pH		Test temperature °C		Conductivity mS/cm		Dissolved O_2 mg/L	
			Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Adult					Test Replicates					
S1	12-Mar-13	C	12.00 ± 0.03	12.04 - 11.96	19.21 ± 0.47	20.50 - 18.60	1.91 ± 0.09	2.07 - 1.73	8.40 ± 0.07	8.52 - 8.22
S2	23-May-12	N	12.00 ± 0.04	12.01 - 11.92	22.84 ± 0.13	23.00 - 22.65	3.78 ± 0.05	3.84 - 3.71	8.70 ± 0.22	9.06 - 8.53
S3	28-May-12	N	11.80 ± 0.04	11.87 - 11.75	21.62 ± 0.18	21.89 - 21.33	2.76 ± 0.10	2.90 - 2.61	8.32 ± 0.18	8.50 - 8.02
Neonate										
S4	8-Nov-13	N	12.00 ± 0.04	12.06 - 11.94	21.57 ± 0.35	21.90 - 20.80	2.76 ± 0.15	2.99 - 2.47	8.25 ± 0.13	8.50 - 8.12
S5	13-Nov-13	N	12.00 ± 0.03	12.05 - 11.97	21.37 ± 0.20	21.70 - 21.10	2.68 ± 0.07	2.77 - 2.47	8.25 ± 0.07	8.45 - 8.18
S6	18-Nov-13	N	12.00 ± 0.02	12.08 - 12.00	21.31 ± 0.29	21.70 - 20.50	2.74 ± 0.10	2.82 - 2.49	8.19 ± 0.16	8.47 - 7.70
S7	4-Feb-14	N	12.00 ± 0.06	12.08 - 11.88	20.26 ± 0.24	20.90 - 19.90	2.52 ± 0.17	2.83 - 2.11	8.23 ± 0.11	8.36 - 8.03
Adult					Control Replicates					
S1	12-Mar-13	C	8.00 ± 0.04	8.05 - 7.94	19.33 ± 0.35	19.82 - 18.89	0.30 ± 0.01	0.31 - 0.29	8.30 ± 0.31	8.81 - 7.95
S2	23-May-12	N	8.10 ± 0.16	8.35 - 7.92	22.86 ± 0.37	23.17 - 22.26	0.97 ± 0.003	0.98 - 0.97	7.36 ± 0.96	8.59 - 6.41
S3	28-May-12	N	8.10 ± 0.04	8.19 - 8.07	21.92 ± 0.49	22.48 - 21.29	0.98 ± 0.05	1.07 - 0.95	7.42 ± 0.20	7.69 - 7.12
Neonate										
S4	8-Nov-13	N	8.30 ± 0.12	8.38 - 8.16	21.60 ± 0.10	21.70 - 21.50	0.30 ± 0.01	0.31 - 0.29	8.28 ± 0.09	8.35 - 8.18
S5	13-Nov-13	N	8.30 ± 0.16	8.54 - 8.17	20.90 ± 0.14	21.00 - 21.70	0.31 ± 0.01	0.32 - 0.30	8.18 ± 0.04	8.23 - 8.14
S6	18-Nov-13	N	8.30 ± 0.15	8.53 - 8.14	20.92 ± 0.19	21.20 - 20.70	0.32 ± 0.02	0.34 - 0.30	8.04 ± 0.21	8.26 - 7.79
S7	4-Feb-14	N	8.20 ± 0.18	8.48 - 7.86	19.81 ± 0.22	20.20 - 19.50	0.35 ± 0.06	0.54 - 0.30	8.38 ± 0.11	8.67 - 8.14

Table 3. Results from the probit regression analysis displaying the predicted mean time to 99% mortality and the lower and upper 95% confidence intervals for adult NZMS in $Ca(OH)_2$ at room temperature at 12 pH (Trial S1). Replicate (Rep) is 5 tea balls, containing 10 NZMS each, at each time interval in the experiment. $P < 0.05$ was indicative of significant differences.

Variable	DF	Chi-square	*P*	Predicted mean LT99 (h)	95% CI (h)
Time	1	34.75	<0.0001	65.2	49.7 – 94.4
Rep	4	0.75	0.95		

Table 4. Results from the probit regression analysis for life stages of NZMS in NaOH at room temperature. Also shown are predicted mean lethal time to 99% mortality for each life stage along with 95% lower and upper confidence intervals. Probit analyses are grouped into two (S2 and S3) trials with adults and four trials (S4, S5, S6, and S7) with neonates. $P < 0.05$ was indicative of significant differences.

Variable	DF	Chi-square	*P*	Predicted mean LT99	95% CI
			Adult (2 trials)		
				H	h
Time	1	14.48	0.0001	39.2	31.9-60.7
Trial	1	0.23	0.63		
Rep	1	0.01	0.92		
			Neonate (4 trials)		
				min	min
Time	1	27.77	<0.0001	5.6	4.1-8.6
Trial	3	3.51	0.32		
Rep	2	0.06	0.97		

Table 5. Summary of pH, temperature, conductivity, and dissolved oxygen in test and control solution of trials with Asian clams. Target test solutions of pH were prepared with either $Ca(OH)_2$ abbreviated C or NaOH abbreviated N. Controls were de-chlorinated well water. Tests and controls are separated by life stage (adult or veligers). Tests and recovery were in fresh water. No conductivity measurements were taken for trial C3.

Trial ID	Test date start	Chemical	pH		Test temperature °C		Conductivity mS/cm		Dissolved O_2 mg/L	
			Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Adult					Test Replicates					
C1	18-Jun-13	C	11.86 ± 0.06	11.93 - 11.78	20.34 ± 1.42	24.40 - 18.28	1.41 ± 0.06	1.48 - 1.29	7.58 ± 0.26	7.86 - 7.16
C2	16-Oct-12	N	11.96 ± 0.09	12.11 - 11.83	20.03 ± 0.39	20.95 - 19.42	3.06 ± 0.32	3.34 - 2.48	7.89 ± 0.10	7.99 - 7.77
C3	25-Apr-13	N	11.91 ± 0.09	12.02 - 11.70	18.53 ± 0.36	19.42 - 17.90	.	.	7.92 ± 0.23	8.17 - 7.25
Veliger										
C6	16-Jul-13	C	11.96 ± 0.01	11.97 - 11.94	22.30 ± 0.21	22.60 - 22.10	1.52 ± 0.02	1.55 - 1.48	7.38 ± 0.03	7.45 - 7.35
C8	29-Jul-13	C	11.82 ± 0.02	11.85 - 11.80	21.90 ± 0.13	22.10 - 21.60	1.07 ± 0.03	1.10 - 1.03	8.44 ± 0.11	8.54 - 8.18
C4	18-Jun-13	C	11.40 ± 0.03	11.42 - 11.35	22.81 ± 0.20	23.20 - 22.60	0.48 ± 0.02	0.51 - 0.44	8.14 ± 0.04	8.21 - 8.08
C7	16-Jul-13	C	11.46 ± 0.02	11.49 - 11.43	21.93 ± 0.17	22.20 - 21.70	0.54 ± 0.02	0.56 - 0.49	6.77 ± 0.07	6.84 - 6.67
C5	1-Jul-13	C	11.17 ± 0.04	11.21 - 11.11	24.86 ± 0.56	25.60 - 24.20	0.46 ± 0.03	0.50 - 0.41	7.75 ± 0.07	7.88 - 7.67
Adult					Control Replicates					
C1	18-Jun-13	C	7.93 ± 0.01	7.94 - 7.91	20.34 ± 1.42	24.40 - 18.28	0.30 ± 0.01	0.31 - 0.29	6.22 ± 0.84	7.35 - 5.35
C2	16-Oct-12	N	7.59 ± 0.10	7.74 - 7.47	20.03 ± 0.39	20.95 - 19.42	0.30 ± 0.002	0.30 - 0.29	7.17 ± 0.36	7.56 - 6.59
C3	25-Apr-13	N	7.95 ± 0.11	8.05 - 7.81	18.53 ± 0.36	19.42 - 17.90	.	.	6.50 ± 0.65	7.23 - 5.27
Veliger										
C6	16-Jul-13	C	8.40 ± 0.05	8.49 - 8.32	22.07 ± 0.31	22.50 - 21.60	0.30 ± 0.01	0.32 - 0.29	8.08 ± 0.11	8.20 - 7.90
C8	29-Jul-13	C	8.12 ± 0.04	8.20 - 8.08	22.01 ± 0.31	22.60 - 21.80	0.30 ± 0.01	0.32 - 0.30	7.91 ± 0.08	8.03 - 7.79
C4	18-Jun-13	C	8.54 ± 0.01	8.55 - 8.53	22.83 ± 0.10	22.90 - 22.70	0.31 ± 0.01	0.32 - 0.29	8.08 ± 0.10	8.17 - 7.96
C7	16-Jul-13	C	8.50 ± 0.11	8.69 - 8.40	21.87 ± 0.20	22.01 - 21.50	0.29 ± 0.04	0.31 - 0.18	8.06 ± 0.03	8.10 - 8.02
C5	1-Jul-13	C	8.35 ± 0.10	8.48 - 8.26	24.72 ± 0.68	25.70 - 24.00	0.31 ± 0.02	0.35 - 0.30	8.18 ± 0.30	8.44 - 7.78

Table 6. Results from the probit regression analysis displaying the predicted mean time to 99% mortality and the lower and upper 95% confidence intervals for adult Asian clams in $Ca(OH)_2$ at room temperature (Trial C1). pH for test solution was 11.8 and for controls was aged water. $P < 0.05$ was indicative of significant differences.

Variable	DF	Chi-square	*P*	Predicted mean LT99 (h)	95% CI (h)
Time	1	1.57	0.21	273.8	.
Rep	1	1.91	0.17		

Table 7. Results from Fisher's exact Chi-square analysis and observed minimum time to mortality for adult Asian clams in $Ca(OH)_2$ (Trial C1) at room temperature. There were two test replicates for each time interval and 10 individuals in each replicate. Treatment variable was control versus test solution at each time interval. pH for test solution was 11.8 and for controls (aged water) pH was neutral.

	Fisher's exact		
Time interval (h)	Two sided *P*	Minimum time to mortality (h)	% dead
150	0.011		
226	0.0016		
275	<0.0001		
342	<0.0001	342	100

Table 8. Summary of time exposure intervals, replicates, number of dead, and total individuals tested in each replicate for tests in $Ca(OH)_2$ at room temperatures using Asian clam veligers. Trials are separated by four pH levels; pH 12 (C6), 11.8 (C8), 11.4 (C4, C7), and 11.2 (C5). For pH 11.4 there were two trials distinguished by Trial 1 (C4) and Trial 2 (C7).

Time exposure (min)	Replicate	Dead	Total	Percent mortality
		12 pH		
60	1	27	27	100
60	2	6	6	100
60	3	18	18	100
90	1	9	9	100
90	2	11	11	100
90	3	6	6	100
120	1	13	13	100
120	2	13	13	100
120	3	11	11	100
		11.8 pH		
60	1	38	50	76
60	2	21	31	68
60	3	34	47	72
90	1	12	18	67
90	2	16	21	76
90	3	17	21	81
120	1	16	18	89
120	2	12	12	100
120	3	9	10	90
		11.4 pH (Trial 1)		
30	1	15	16	94
30	2	14	14	100
30	3	71	84	85
30	4	16	20	80
60	1	15	15	100
60	2	20	20	100
60	3	17	17	100
60	4	10	10	100

Time exposure (min)	Replicate	Dead	Total	Percent mortality
11.4 pH (Trial 2)				
60	1	18	18	100
60	2	8	8	100
60	3	9	10	90
90	1	6	6	100
90	2	23	23	100
90	3	22	22	100
120	1	14	14	100
120	2	14	14	100
120	3	23	23	100
11.2 pH				
60	1	6	16	38
60	2	18	71	25
60	3	11	45	24
75	1	17	19	89
75	2	16	17	94
75	3	4	7	57
90	1	16	17	94
90	2	10	14	71
90	3	4	4	100

Table 9. Model summary and results from the GLM procedure with ranking of LSMEANs for 60 minute intervals for veliger Asian clams in $Ca(OH)_2$ at room temperature. Dependent variable is mortality response at different levels of pH exposure: 12 (C6), 11.8 (C8), 11.4 (C4, C7), and 11.2 (C5). This summary includes all five veliger trials.

Type III Sum of Squares Model Fit Parameters

Source	DF	Sum of squares	Mean square	F value	*P*-value
ph	3	11720.39	3906.79	202.91	<0.0001

			LSMEANS Number			
pH	Mort LSMEASE	LSMEANS Number	1	2	3	4
11.2	28.67	1		<0.0001	<0.0001	<0.0001
11.4	98.57	2	<0.0001		<0.0001	0.6455
11.8	71.67	3	<0.0001	<0.0001		<0.0001
12	100	4	<0.0001	0.6455	<0.0001	

Table 10. Model summary and results from the GLM procedure with LSMEAN mortality for veliger Asian clams in $Ca(OH)_2$ at room temperature at 90 min time interval. Dependent variable is mortality response at different levels of pH exposure: 12 (C6), 11.4 (C7), 11.2 (C5).

Type III Sum of Squares Model Fit Parameters

Source	DF	Sum of squares	Mean square	F value	*P*-value
Ph	2	272.22	136.11	1.74	0.2531

			LSMEANS Number		
pH	Mort LSMEASE	LSMEANS Number	1	2	3
11.2	88.333	1		0.1571	0.1571
11.4	100	2	0.1571		1
12	100	3	0.1571	1	

Table 11. Results from Fisher's exact chi-square analysis of the proportion live and dead in test versus controls at three time intervals of exposure for veliger Asian clams in $Ca(OH)_2$ at room temperature. Each time interval had three test replicates. pH treatment was 11.2 (Trial C5).

Time interval (min)	Individuals/rep	Sample size	Fisher's exact Two sided *P*	Minimum time to mortality (min)	% dead
60	16-71	454	0.0749		
75	7-19	606	<0.0001		
90	4-17	298	<0.0001	90	71-100

Table 12. Results for Fisher's exact chi-square analysis of the proportion live and dead in test versus controls at 4 time intervals of exposure for two trials with veliger Asian clams in $Ca(OH)_2$ at room temperature. pH level was 11.4 (Trials C4 and C7).

					Fisher's exact			
Trial	Time interval (min)	# Reps	# Individuals/rep	Sample size	Two sided *P*	Trial	Minimum time to mortality (min)	% dead
C4	30	4	14-84	187	<0.0001			
C4	60	4	10-20	98	<0.0001	C4	60	100
C7	60	3	8-18	107	<0.0001			
C7	90	3	6-23	159	<0.0001	C7	90	100
C7	120	3	14-23	199	<0.0001			

Table 13. Results from Fisher's exact chi-square analysis of proportion live and dead in tests at 60 min time interval for veliger Asian clams in $Ca(OH)_2$ at room temperature in 11.4 pH (Trials C4 and C7).

		Fisher's exact			
Time interval (min)	Sample size	Two sided *P*	Trial	Minimum time to mortality (min)	% dead
60	98	0.3673	C4	60	100
			C7	90	100

Table 14. Results Fisher's exact chi-square analysis of the proportion live and dead in test versus controls at three time intervals of exposure for veliger Asian clams in $Ca(OH)_2$ at room temperature at 11.8 pH (Trial C8).

Time interval (min)	Sample size	Fisher's exact Two sided *P*	Minimum time to mortality (min)	% dead
60	365	<0.0001		
90	196	<0.0001		
120	186	<0.0001	120	88-100

Table 15. Results from Fisher's exact chi-square analysis and observed minimum time to mortality of veliger Asian clams in $Ca(OH)_2$ at room temperature. Treatment variable is control versus test solution response at each time interval. pH of test solution is 12 (Trial C6).

		Fisher's exact		
Time interval (min)	Sample size	Two sided *P*	Minimum time to mortality (min)	% dead
60	95	<0.0001	60	100
90	91	<0.0001		
120	117	<0.0001		

Table 16. Results from the probit regression analysis of mean time to mortality for adult Asian clams in NaOH (Trial C2 and C3) at room temperature. Also shown are predicted mean lethal time to 99% mortality along with 95% lower and upper confidence intervals. P < 0.05 was indicative of significant differences.

Variable	DF	Chi-square	*P*	Predicted mean LT99	95% CI
				H	h
Time	1	65.6	<0.0001	214.6	189.2–251.1
Trial	1	0.07	0.79		
Rep	3	1.4	0.71		

Table 17. Summary of pH, temperature, conductivity, and dissolved oxygen in test and control trials with quagga mussels. Target solutions of pH were prepared with either $Ca(OH)_2$ abbreviated C or NaOH abbreviated N. Control solutions were de-chlorinated well water. Tests and controls are separated by life stage (adult or veligers) Tests and recovery were in fresh water.

Trial ID	Test date start	Chemical	pH		Test temperature °C		Conductivity mS/cm		Dissolved O_2 mg/L	
			Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Adult					Test Replicates					
Q1	18-Nov-12	C	11.98 ± 0.03	12.02 - 11.95	19.64 ± 0.51	20.08 - 18.92	3.91 ± 0.06	3.99 - 3.85	8.16 ± 0.38	8.56 - 7.69
Q2	29-May-12	N	11.91 ± 0.02	11.94 - 11.88	21.00 ± 0.21	21.36 - 20.78	3.01 ± 0.04	3.05 - 2.95	8.46 ± 0.11	8.57 - 8.32
Q3	18-Nov-12	N	11.99 ± 0.05	12.07 - 11.95	19.97 ± 0.67	20.47 - 19.01	4.07 ± 0.14	4.27 - 3.97	9.33 ± 0.15	9.50 - 9.17
Q4	25-May-12	N	11.92 ± 0.08	11.97 - 11.80	16.75 ± 0.30	16.90 - 16.30	3.05 ± 0.22	3.26 - 2.76	8.12 ± 0.69	8.78 - 7.15
Juvenile										
Q7	18-May-12	N	11.98 ± 0.06	12.05 - 11.91	21.12 ± 0.79	21.93 - 20.07	2.97 ± 0.10	3.06 - 2.8	9.03 ± 0.18	9.27 - 8.74
Adult					Control Replicates					
Q1	18-Nov-12	C	7.99 ± 0.17	8.17 - 7.81	19.50 ± 0.65	19.97 - 18.54	0.88 ± 0.003	0.88 - 0.88	8.10 ± 0.99	9.29 - 6.88
Q2	29-May-12	N	7.90 ± 0.10	8.01 - 7. 73	21.00 ± 0.44	21.86 - 20.70	0.97 ± 0.01	0.99 - 0.96	6.78 ± 0.62	7.29 - 5.55
Q3	18-Nov-12	N	7.98 ± 0.19	8.25 - 7.81	19.59 ± 0.72	20.37 - 18.63	0.88 ± 0.01	0.88 - 0.87	7.98 ± 0.95	9.19 - 6.97
Q4	25-May-12	N	7.98 ± 0.25	8.14 - 7.70	17.37 ± 0.02	17.39 - 17.35	0.98 ± 0.02	1.01 - 0.96	4.77 ± 2.26	7.30 - 2.20
Juvenile										
Q7	18-May-12	N	8.08 ± 0.32	8.53 - 7.74	21.49 ± 0.97	22.41 - 20.02	0.93 ± 0.02	0.95 - 0.90	7.75 ± 0.89	8.81 - 6.70

Table 18. Results from the probit regression analysis and estimated mean time to 99% mortality with 95% lower and upper confidence intervals for adult quagga mussels in $Ca(OH)_2$ at room temperature (Trial Q1).

Variable	DF	Chi-square	*P*	Predicted mean LT99 (h)	95% CI (h)
Time	1	4.9	0.03	7.9	4.8 – 47.9
Rep	2	0.18	0.91		

Table 19. Results from the Fisher's exact chi-square analysis of the proportion live and dead in test versus controls at four time intervals of exposure for adult quagga mussels in $Ca(OH)_2$ at room temperature at 12 pH (Trial Q1).

		Fisher's exact		
Time interval (h)	Sample size	Two sided *P*	Minimum time to mortality (h)	% dead
2	38	<0.0001		
6	39	<0.0001		
15	40	<0.0001	15	100
42	40	<0.0001		

Table 20. Results from Fisher's exact chi-square analysis of the proportion live and dead in test replicates between two trials at time intervals 2 min and 5 min for veliger quagga mussels in $Ca(OH)_2$ at cooler temperature (Trial Q13) and room temperature (Trial Q11).

Time interval (min)	Sample size	Fisher's exact Two sided *P*	Test temp	Minimum time to mortality (min)	% dead
2	378	<0.0001	Room	10	100
5	362	0.0032	Cool	10	100

Table 21. Results from the probit regression analysis and estimated mean time to 99% with 95% lower and upper confidence intervals for veliger quagga mussels in $Ca(OH)_2$ at cooler temperature (Trial Q13) and room temperature (Trial Q11).

Trial	Target test temp °C	Variable	DF	Chi-square	*P*	Test temp	Predicted mean LT99 (min)	95% CI (min)
Q13	Cool	Time	1	206.3	<0.0001	15	6.3	5.7 - 7.
Q13	Cool	Rep	3	0.71	0.87			
Q11	Room	Time	1	21.1	<0.0001	20	6.7	5 - 10.8.
Q11	Room	Rep	3	4.4	0.22			

Table 22. Results from the Fisher's exact chi-square analysis of the proportion live and dead in test versus controls between two trials at four time intervals of exposure for veliger quagga mussels in $Ca(OH)_2$ for a trial at room temperature (Trial Q13) and a trial at cooler temperature (Trial Q11).

Trial ID	Time interval (min)	Sample size	Fisher's exact Two sided *P*	Test temp	Minimum time to mortality (min)	% dead
Q11	2	258	<0.0001			
Q11	5	214	<0.0001	Room	5	90-100
Q11	10	115	<0.0001	Room	10	100
Q11	20	183	<0.0001			
Q13	2	323	<0.0001			
Q13	5	270	<0.0001	Cool	5	86-90
Q13	10	182	<0.0001	Cool	10	10
Q13	20	94	<0.0001			

Table 23. Results from the probit regression analysis for two adult quagga mussel trials in NaOH at room temperature (trial Q2 and Q3). $P < 0.05$ was indicative of significant differences.

Variable	DF	Chi-square	*P*	Predicted mean LT99 (h)	95% CI (h)
Time	1	21.2	<0.0001	33.7	25.2 – 53.2
Trial	1	2.5	0.11		
Rep	2	0.37	0.83		

Table 24. Results from Fisher's exact chi-square analysis of the proportion live and dead in test versus controls in two trials at nine time intervals of exposure for adult quagga mussels in NaOH at room temperature (trials Q2 and Q3). P<0.05 was indicative of significant differences.

Trial	Time interval (h)	# Reps	# Individuals/rep	Sample size	Fisher's exact Two sided *P*	Trial	Minimum time to mortality (h)	% dead
Q3	2	3	9-10	40	0.556			
Q3	6	3	9-10	38	0.156			
Q3	15	3	9-10	39	0.002			
Q3	42	3	9-10	39	<0.0001	1	42	100
Q2	10	2	4-5	12	0.491			
Q2	12	2	4-5	13	0.105			
Q2	14	2	4-5	12	0.010			
Q2	15	2	4-5	12	0.081			
Q2	16	2	4-5	12	0.010			
Q2	18	2	4-5	12	0.010	2	18	75-100

Table 25. Results from Fisher's exact chi-square analysis of the proportion live and dead in test versus controls at four time intervals of exposure for adult Quagga Mussels in NaOH at cooler test temperature (Trial Q4). There were three replicates for each time interval and only 2 individuals tested in each replicate. Sample size in each time interval is 8.

	Fisher's exact		
Time interval (h)	Two sided *P*	Minimum time to mortality (h)	% dead
48	0.1071		
72	0.0357	72	100
96	0.0357		
120	0.0357		

Table 26. Results from the probit regression analysis for juvenile quagga mussels in NaOH at room temperature (trial Q7). Also shown are predicted mean lethal time to 99% mortality along with 95% lower and upper confidence intervals. $P < 0.05$ was indicative of significant differences.

Variable	DF	Chi-square	*P*	Predicted mean LT99 (h)	95% CI (h)
Time	1	36.5	<0.0001	12.6	10.4 - 16.5
Rep	3	17.6	0.0005		

Table 27. Results from Fisher's exact chi-square analysis of the proportion live and dead in test versus controls at six time intervals of exposure for juvenile quagga mussels in NaOH at room temperature (trial Q7). There are four test replicates for each time interval and five individuals in each replicate. Sample size for all intervals is 25.

	Fishers exact		
Time interval (h)	Two sided *P*	Minimum time to mortality (h)	% dead
1	1		
2	0.2887		
4	0.0391		
6	0.0047		
8	0.0011		
10	<0.0001	10	80-100

Table 28. Results from the chi-square analysis with Fisher's exact test of the proportion live and dead in test versus controls at four time intervals of exposure in two trials for veliger quagga mussels. One trial was conducted in NaOH at cooler (trial Q12) temperature and the other conducted at room temperature (trial Q10). Each time interval had four test replicates.

				Fisher's exact			
Trial ID	Time interval (min)	Individuals /rep	Sample size	Two sided *P*	Test temp °C	Minimum time to mortality (min)	% dead
Q12	5	16-28	151	<0.0001			
Q12	10	12-33	123	<0.0001			
Q12	20	11-23	123	<0.0001			
Q12	30	11-18	94	<0.0001	Cool	30	100
Q10	5	10-20	95	0.0014			
Q10	10	20-26	200	<0.0001	Room	10	100
Q10	20	15-23	196	<0.0001			
Q10	30	19-21	174	<0.0001			

Table 29. Results from the probit regression analysis, predicted mean lethal time to 99% mortality, and lower and upper 95% confidence intervals for veliger quagga mussels in NaOH at cooler test temperature (trial Q12).

Variable	DF	Chi-square	*P*	Predicted mean LT99 (min)	95% CI (min)
Time	1	29.74	<0.0001	20.7	16.4 – 28.5
Rep	3	3.43	0.33		

Table 30. Results from the Fisher's exact chi-square analysis of the proportion live and dead in tests replicates at three time intervals of exposure between two trials with veliger quagga mussels in NaOH at cooler temperature (trial Q12) and room temperature (trial Q10).

		Fisher's exact			
Time interval (min)	Sample size	Two sided *P*	Test temp	Minimum time to mortality (min)	% dead
5	139	0.2991	Room	10	100
10	179	<0.0001	Cool	30	100
20	142	0.2412			

Table 31. Summary of pH, temperature, conductivity, and dissolved oxygen in test and controls of trials with quagga mussels tested in target solutions of pH prepared with either $Ca(OH)_2$ abbreviated C, NaOH abbreviated N, or neutral pH abbreviated H2O. Tests and controls are separated by life stage (adult or veligers). All tests were conducted in 15 ppt brackish water solutions. Recovery for adult trials was fresh water but for juveniles' replicates were split and 2 replicates were recovered in fresh water while the other two replicates were recovered in 15 ppt brackish water. Controls used fresh acclimated raceway water, except the control for trial Q9 used brackish water (15ppt).

Trial ID	Test date start	Chemical	pH		Test temperature °C		Conductivity mS/cm		Dissolved O_2 mg/L	
			Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Adults					Test Replicates					
Q5	21-Nov-12	N	11.9 ± 0.02	11.92 - 11.87	21.48 ± 0.80	22.53 - 20.62	24.76 ± 0.04	24.80 - 24.70	8.93 ± 0.13	9.02 - 8.73
Q6	21-Nov-12	H2O	8.43 ± 0.18	8.69 - 8.30	21.25 ± 0.96	22.49 - 20.30	24.35 ± 0.19	24.5 - 24.07	9.50 ± 0.31	9.78 - 9.10
Juvenile										
Q8	27-May-12	N	11.96 ± 0.02	11.98 - 11.94	21.19 ± 0.14	21.31 - 20.98	25.10 ± 0.19	25.27 - 24.88	8.16 ± 0.11	8.29 - 8.02
Adults					Control Replicates					
Q5	21-Nov-12	N	8.49 ± 0.23	8.81 - 8.29	20.96 ± 0.65	21.90 - 20.42	0.91 ± 0.04	0.97 - 0.89	8.80 ± 0.89	9.78 - 7.67
Q6	21-Nov-12	H2O	8.39 ± 0.12	8.51 - 8.23	20.95 ± 0.70	21.99 - 20.51	0.89 ± 0.01	0.90 - 0.89	9.03 ± 0.81	9.88 - 8.03
Juvenile										
Q9	27-May-12	N	8.72 ± 0.17	8.84 - 8.53	20.98 ± 0.39	21.43 - 20.57	24.14 ± 0.05	24.2 - 24.08	8.59 ± 0.18	8.78 - 8.4
Q8	27-May-12	N	8.68 ± 0.16	8.79 - 8.50	21.06 ± 0.55	21.72 - 20.42	1.19 ± 0.11	1.32 - 1.10	7.80 ± 0.36	8.20 - 7.50

Table 32. Results from the probit regression analysis and estimated mean time to 99% mortality with 95% lower and upper confidence intervals for adult quagga mussels in brackish (15ppt) NaOH at room temperature. One trial was brackish water with neutral pH (trial Q6) and the other trial was brackish solution with 12 pH NaOH (trial Q5). Mortality response in trials was not significantly different and trials were run in probit together.

Variable	DF	Chi-square	*P*	Predicted mean LT99 (h)	95% CI (h)
Time	1	43.5	<0.0001	14.3	11.5 - 19
Trial	1	3.2	0.07		
Rep	2	1.9	0.38		

Table 33. Results from Fisher's exact chi-square analysis of the proportion live and dead in tests at 2 h time interval between two trials, one with juvenile (trial Q8) and one with adult quagga (trial Q5) mussels in brackish NaOH with fresh water recovery at room temperature.

	Fisher's exact			
Time interval (h)	Two sided P	Test	Minimum time to mortality (h)	% dead
2	<0.0001	Juv	2	100
		Adult	10	71-100

Table 34. Results from the Fisher's exact chi-square analysis of the proportion live and dead in test versus controls at four time intervals of exposure for juvenile quagga mussels in brackish NaOH at room temperature (trial Q8). Tests recovered in fresh water.

	Fisher's exact		
Time interval (h)	Two sided *P*	Minimum time to mortality (h)	% dead
1	0.491		
2	0.002	2	100
4	0.002		
6	0.002		

Table 35. Results from the Fisher's exact chi-square analysis of the proportion live and dead in tests at 1 h time interval between two trials; one with juvenile quagga mussels in brackish NaOH with fresh water recovery (trial Q8) and the other with brackish water recovery (trial Q9) at room temperature.

		Fisher's exact			
Time interval (h)	Sample size	Two sided *P*	Test recovery	Minimum time to mortality (h)	% dead
1	16	0.03	fresh	2	100
			brackish	1	100

Table 36. Summary of probit model estimates of mean hours to 99% mortality (LT99), and upper 95% confidence interval for adults of three species and two test compounds used to elevate pH to 12 at temperature from 18 to 23°C. All trials were conducted in fresh water.

Species	Chemical	Estimated mean LT99 (h)	Upper 95% CI (h)
New Zealand mudsnail			
	Ca(OH)2	65.2	94.4
	NaOH	39.2	60.7
Asian clams			
	NaOH	214.6	251.1
Quagga mussel			
	Ca(OH)2	7.9	47.9
	NaOH	33.7	53.2

Table 37. Summary of observed time to 100% mortality and probit model estimates of upper 95% confidence interval in minutes of dispersal life stage of two species and two test compounds used to elevate pH to 12. Trials tested between 19 and 21°C were designated as "room" temperature and trials tested at 15°C were designated as "cool." All trials were conducted in fresh water. Astrick represents times that could not be estimated due to rapid mortality.

Species	Chemical	Temperature	Observed time to 100% mortality (min)	Upper 95% CI time (min)
New Zealand mudsnail				
	NaOH	Room	6-8	8.6
Quagga mussel				
	Ca(OH)2	Room	10	10.8
	Ca(OH)2	Cool	10	7
	NaOH	Room	10	*
	NaOH	Cool	30	28.5

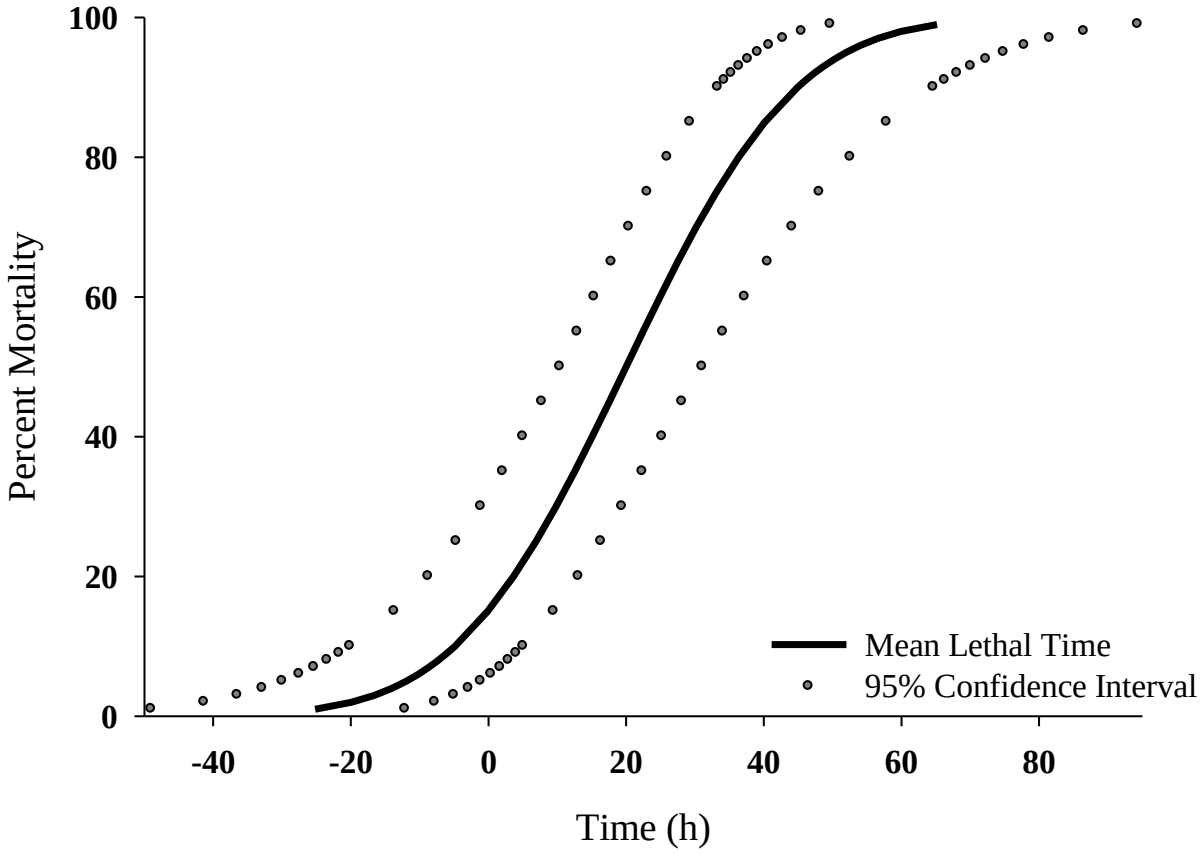

Figure 1. Probit estimate of mean (solid line) and 95% confidence intervals (dashed lines) of survival response of adult NZMS exposed to elevated pH using $Ca(OH)_2$ at room temperature (trial S1).

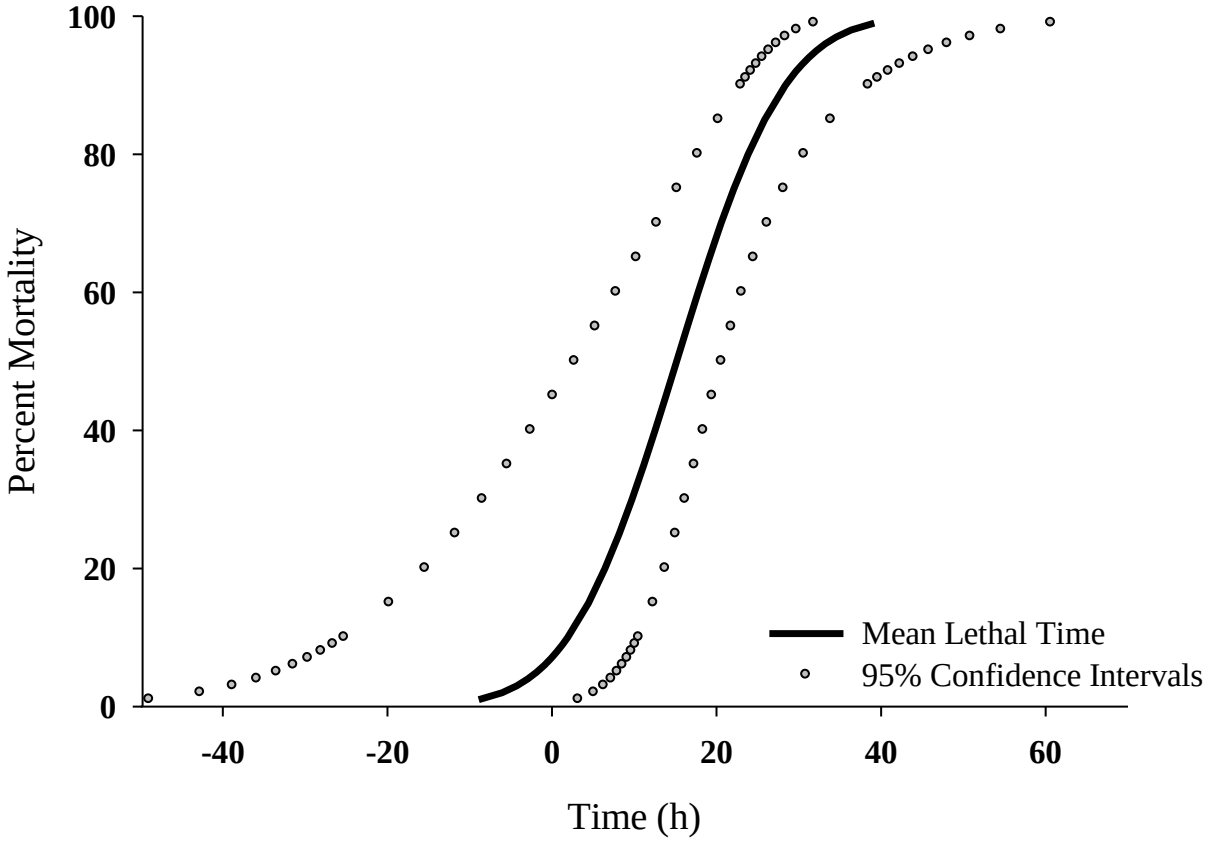

Figure 2. Probit estimate of mean (solid line) and 95% confidence intervals (dashed lines) of the survival response of adult NZMS exposed to elevated pH using NaOH at room temperature (trial S2 and S3).

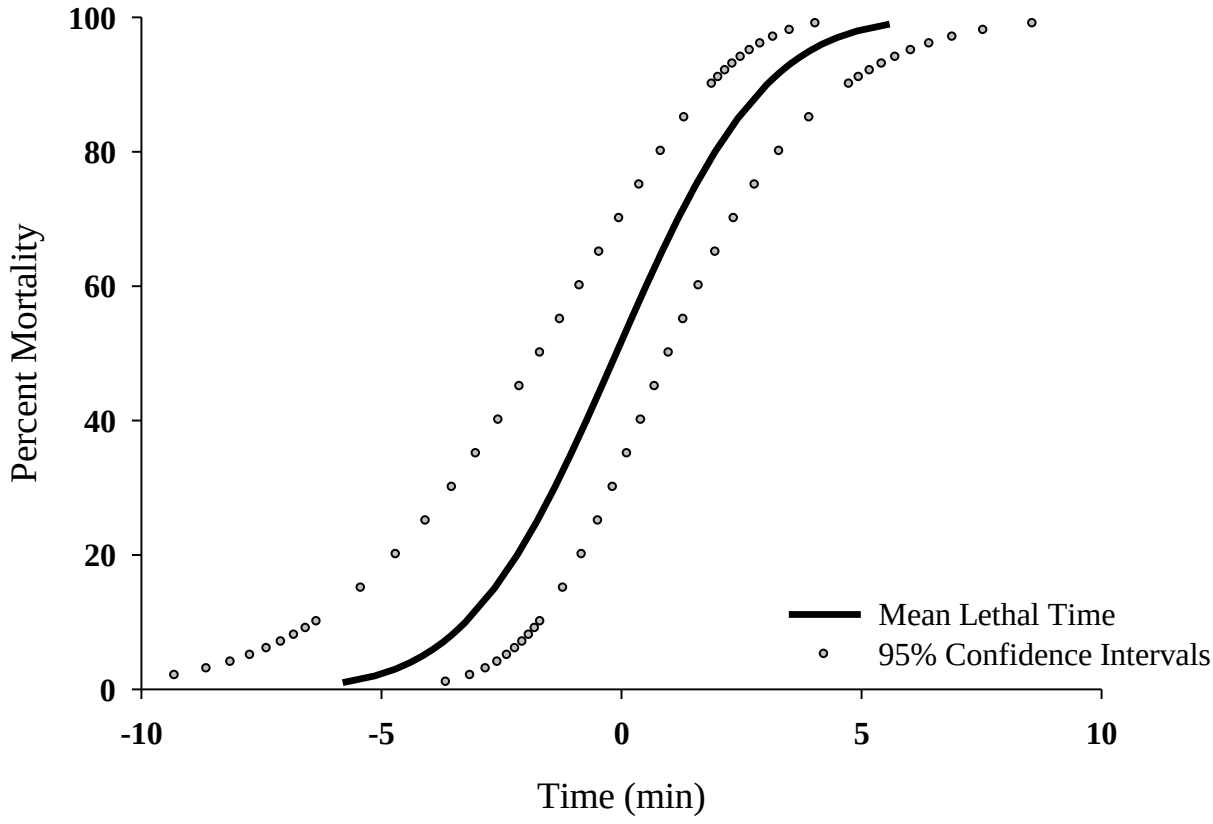

Figure 3. Probit estimate of mean (solid line) and 95% confidence intervals (dashed lines) of the survival response of NZMS neonates exposed to elevated pH using NaOH at room temperature. All four neonate trials were included in this graph (trial S4, S5, S6, and S7).

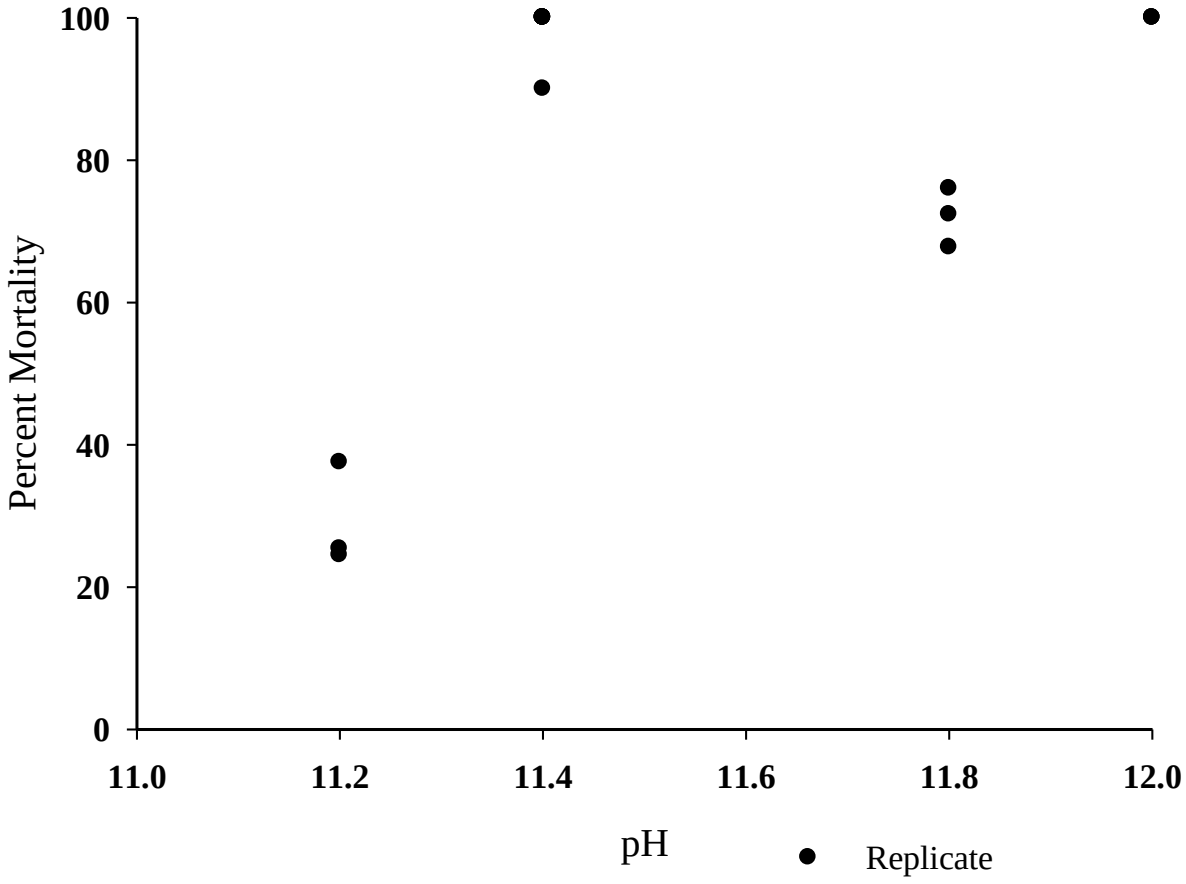

Figure 4. Percent mortality of Asian clam veligers in $Ca(OH)_2$ at room temperature in all replicates for each of the different trials at the 60 min time interval. Trials are designated as 11.2 (C5), 11.4 (C4, C7), 11.8 (C8), and 12 (C6) pH.

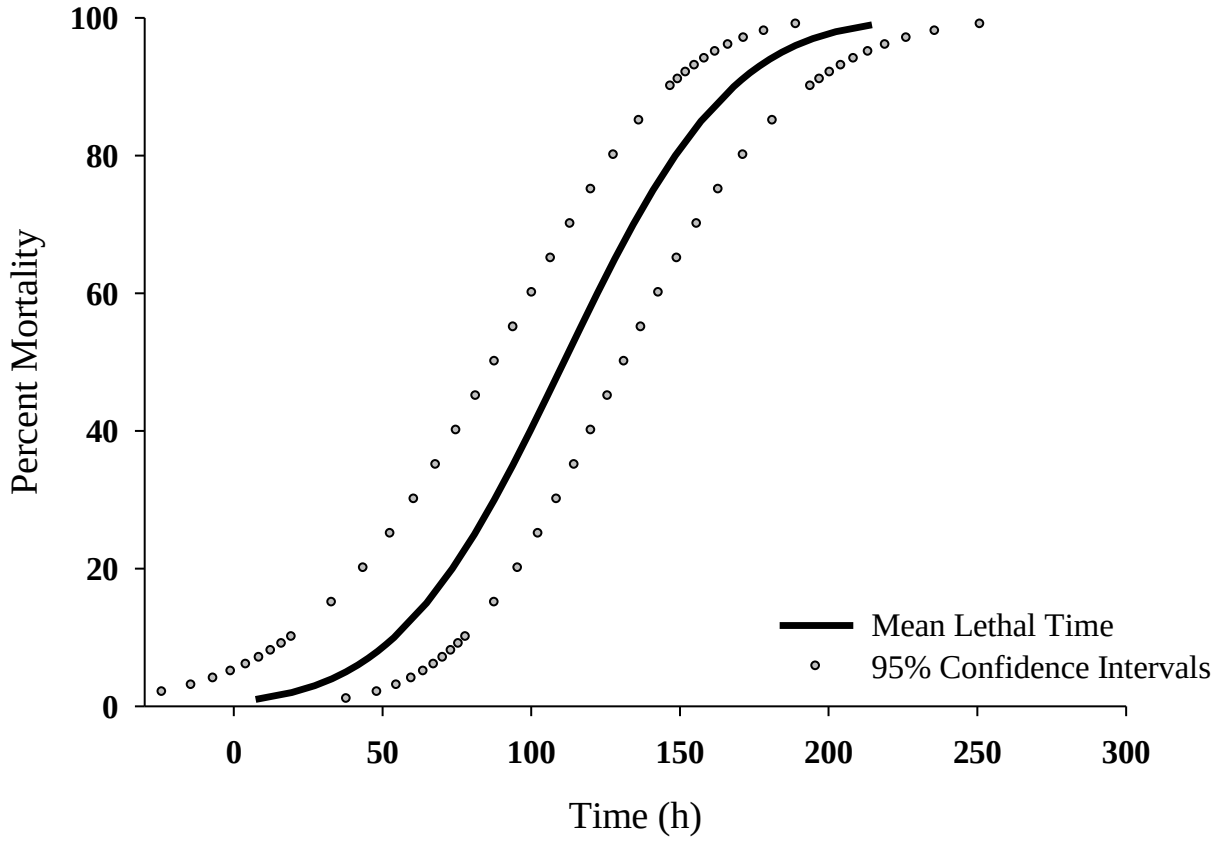

Figure 5. Probit estimate of mean (solid line) and 95% confidence intervals (dashed lines) of the survival response of two trials (C2 and C3) with Asian clam adults exposed to elevated pH 12 using NaOH at room temperature.

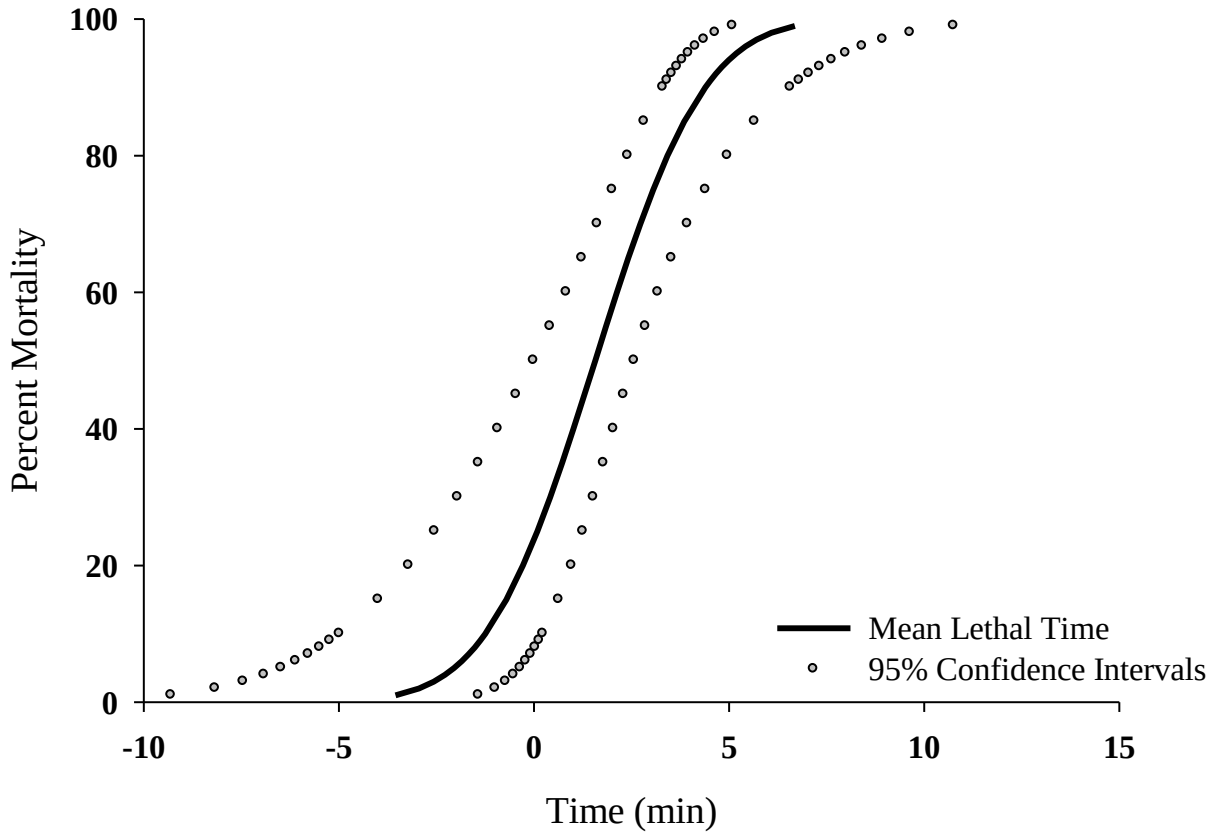

Figure 6. Probit estimate of mean (solid line) and 95% confidence intervals (dashed lines) of the survival response of quagga mussel veligers exposed to elevated pH 12 using $Ca(OH)_2$ at room temperature (Q11).

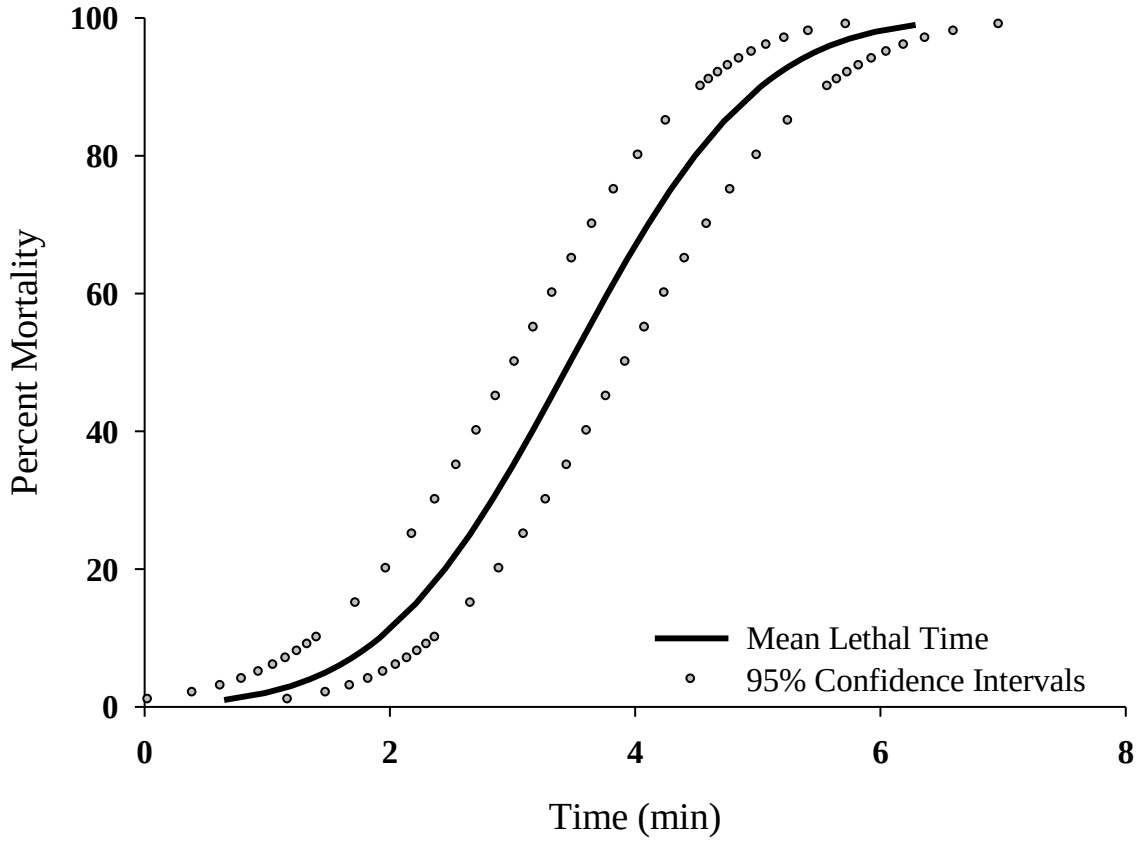

Figure 7. Probit estimate of mean (solid line) and 95% confidence intervals (dashed lines) of the survival response of quagga mussel veligers exposed to elevated pH 12 using $Ca(OH)_2$ at room temperature (Q13)

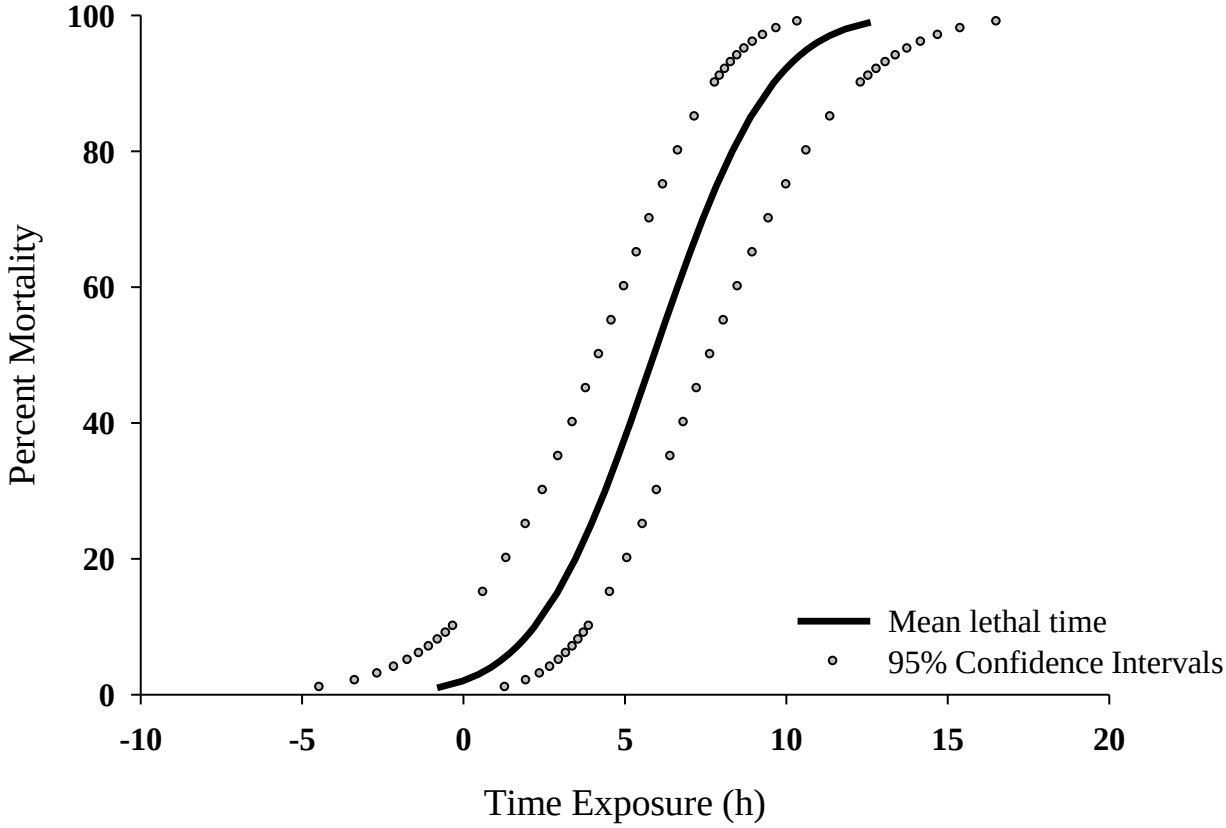

Figure 8. Probit estimate of mean (solid line) and 95% confidence intervals (dashed lines) of the survival response of one trial (Q7) with quagga mussel juveniles exposed to elevated pH using NaOH at room temperature.

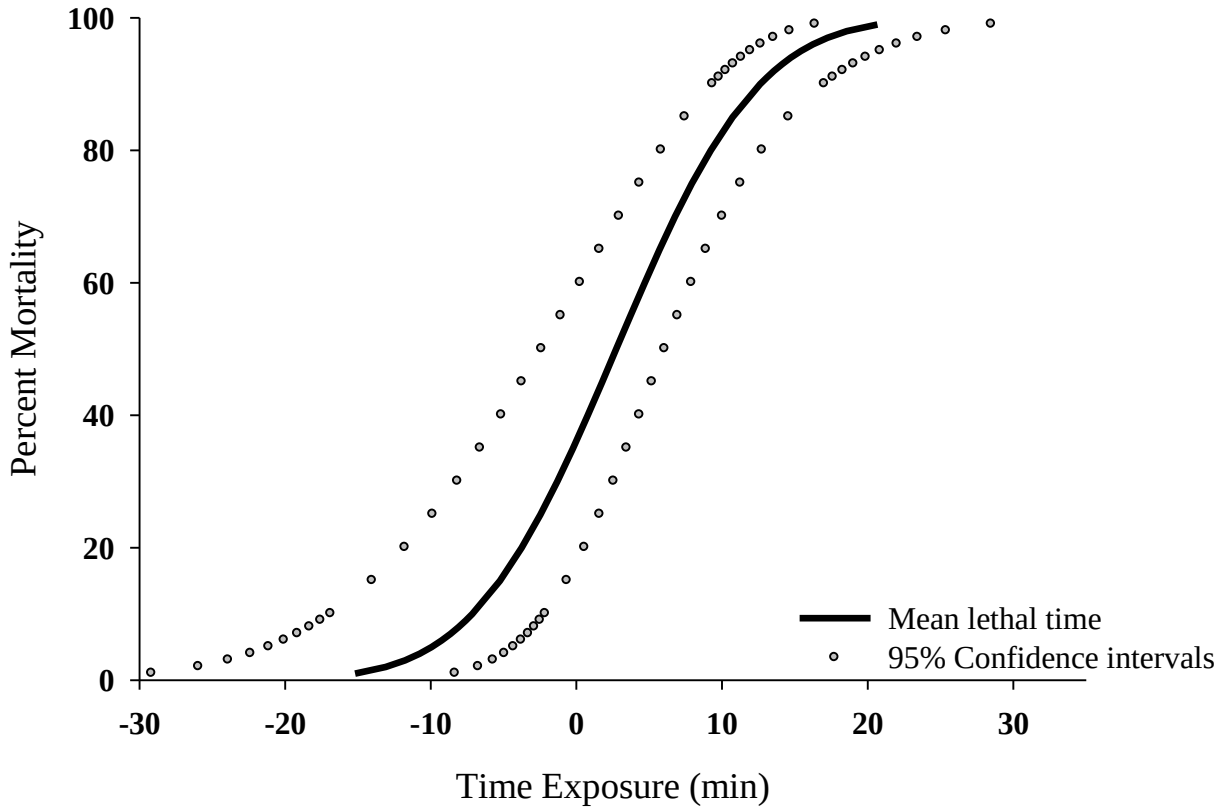

Figure 9. Probit estimate of mean (solid line) and 95% confidence intervals (dashed lines) of the survival response of one trial (Q12) with quagga mussel veligers exposed to elevated pH using NaOH at cool temperatures.

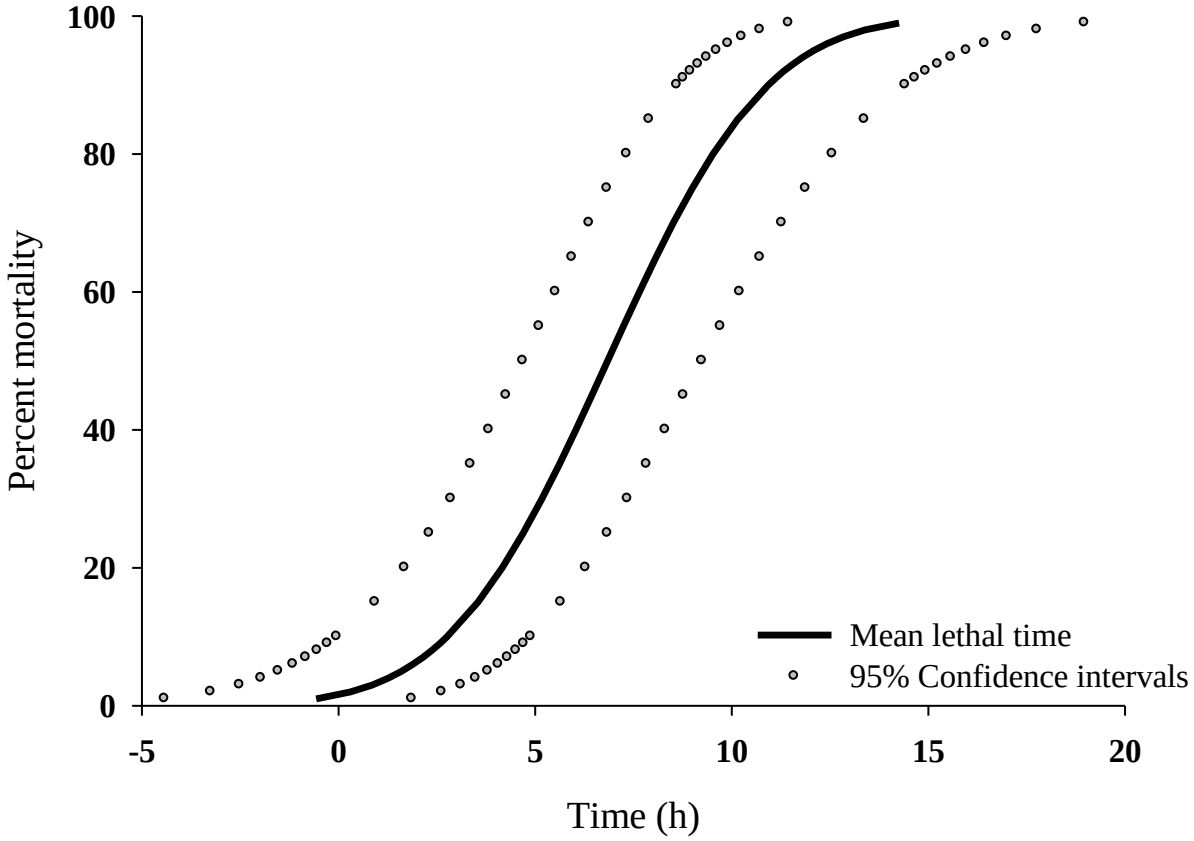

Figure 10. Probit estimate of mean (solid line) and 95% confidence intervals (dashed lines) of the survival response of two trials (Q5 and Q6) with quagga mussel adults exposed to 15 ppt brackish water as well as brackish NaOH at room temperature.

Appendix 1

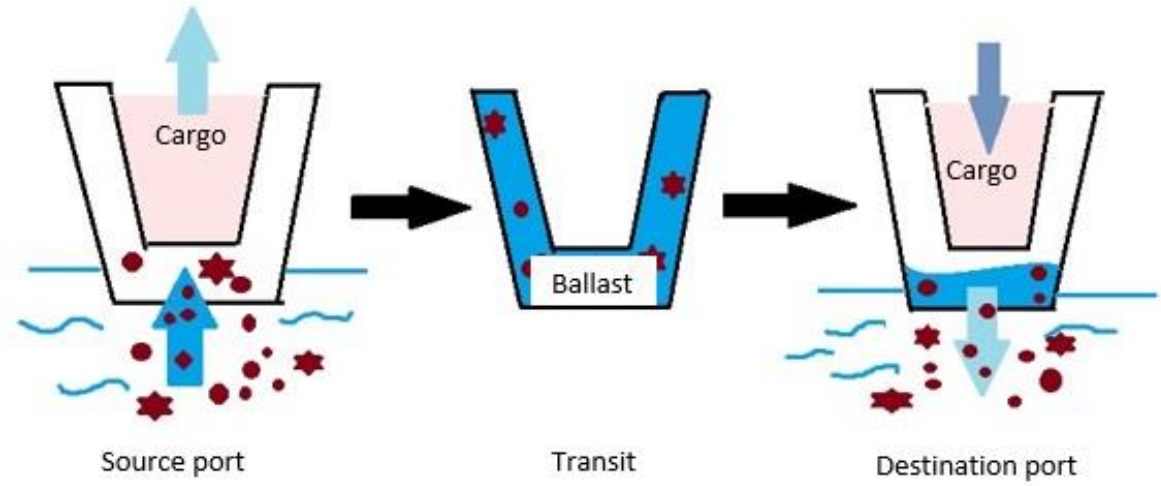

Image is adapted from original image by Chan et al (2013).

Top image shows function of a ballast tank. At the source port as supplies are unloaded from the cargo hold water, including living organisms in the water, are pumped into the ballast tank to counter act the loss in weight from the cargo hold. During transit the cargo hold is empty and the ballast tank is now full of water and organisms from source port. This helps to maintain the structural integrity of the ship during voyage. At the destination port as supplies are loaded back into the cargo hold, water and organisms from the ballast tanks are released into the surrounding area to maintain ship stability.

Appendix 2

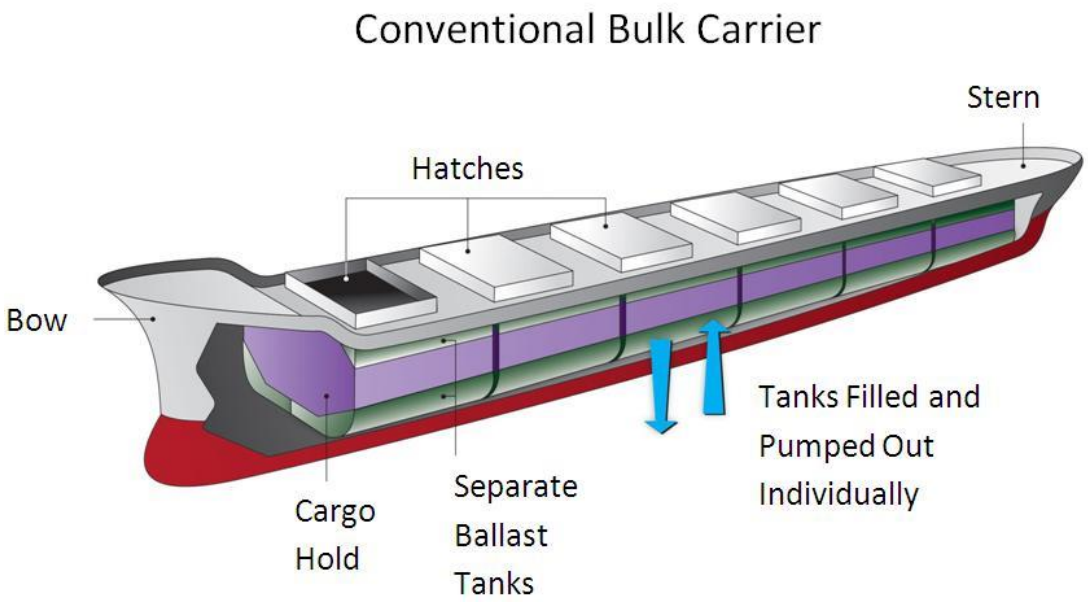

Image is adapted from original image in article by Rosalind Echols (2013).

Image shows location of ballast tanks relative to the cargo hold on a conventional bulk carrier. Ballast tanks are filled with water and pumped out individually to help maintain balance and structural integrity of the carrier during transit and loading/offloading of supplies from the cargo hold.

Appendix 3

Inducing Asian clams to release veligers/gametes in laboratory settings using cross fertilization technique

Materials:

1. Petri dish
2. Dissecting needle
3. Scalpel
4. 20-30 adult Asian clams
5. Disposable plastic pipettes
6. De-chlorinated well water
7. Air stones
8. 5.7 L holding container
9. Dissecting microscope/lamp
10. Black counter or table top.

Introduction:

The ability to induce adult Asian Clams to release gametes or developed veligers aids researchers by providing them with a viable population for testing and rearing. To our knowledge no known literature exists on how to induce Asian clams to spawn in the laboratory setting. Several techniques were tried such as temperature shock, toxicity exposure, removal of gonads directly from the specimen and the cross fertilization technique. The most successful method was the cross fertilization technique and is the focus of this Standard Operating Procedure, however, the other methods are described briefly below.

The temperature shock process consisted of placing between 5-10 adult Asian clams in a 1.8 L container filled half way with de-chlorinated well water and placing them onto a hot plate.

Water temperature was allowed to reach the upper thermal tolerance level of Asian clams (32-35°C). Asian clams were left at this elevated temp for 2 hours after which the water was drained and 15°C water was poured over the clams. Adult clams were observed for 48 hours. This technique did not seem to have any effect on the clams or result in release of veligers.

For the toxicity shock, a group of adult Asian clams was placed into elevated ph (12) solution using either sodium hydroxide or calcium hydroxide for 24 hours. The next day the clams were removed and allowed to recover in fresh de-chlorinated water at 20°C for 48 hours. We never saw the clams spawn after this treatment but rarely on the 3rd or 4th day after exposure some clams did release veligers. Whether or not this is a result of elevated pH exposure is not known and should be further investigated.

Removal and rearing of the ovaries and sperm directly from the visceral mass of a freshly opened adult calm did not appear to be very successful. After the eggs were removed directly from the clam they were placed into de-chlorinated well water in a 1.8 L container with supplemented oxygen. The eggs were observed to see if they were viable and in all cases after a few days, the eggs appeared fuzzy and eventually disintegrated and disappeared from the water. We believe this is because the eggs were not fertilized prior to being placed in the new container.

The most successful method to induce the release of viable veligers was what we call the cross fertilization technique. On two separate occasions (different year dates and different times of season) this method proved to be successful on inducing two separate populations. One population was induced in the summer of 2013 from clams from the Bruhno River in

southern Idaho and the second instance occurred in the winter of 2014 with clams from Lake Pend Oreille in north Idaho. We are unsure of the exact mechanisms at play but believe it to be pheromones. The methods of this technique are described in detail below. We recommend performing this technique on a black table top so that veligers can be more easily located in the containers.

Methods:

System setup

1. Place between 20-50 adult Asian clams into a clean 5.7 L container filled halfway with de-chlorinated well water
   NOTE: Do not place any sand or sediments of any kind into the container with the clams that will compromise the visibility of locating the veligers.
2. Acclimate and keep Asian clams at approximately 20°C for the duration of the release
3. Place small air stones in container to provide supplemented aeration
4. Exchange the water in the container every other day until the clams begin to spawn
   NOTE: Exchanging water during veliger release will result in loss of veligers from the system and also disrupt releasing adults.
5. Keep clams at 20°C for 2-3 days before starting the spawning procedures

Inducing

6. Prior to inducing clams, change water in the container and remove air stones
7. Remove a single adult Asian clam from the container and place onto a petri dish
8. Open the clam using a dissecting needle and scalpel (if needed) on the side the siphons are located on
   NOTE: Be careful not to damage tissues and organs inside during this process
9. Locate the visceral mass, usually found towards the back of the clam by the umbo, opposite the side of the siphons
10. Cut into the visceral mass containing the gonads
11. Wash the gonads out of the clam using some de-chlorinated water and a plastic pipette and allow the gonads to flow into the bottom of the petri dish
    NOTE: The visceral sack containing the gonads is next to the digestive glad and so some contents from the digestive glad may get mixed into the ovaries in the petri dish. This does not seem to effect the success of initiating spawning in the other adults.

NOTE: One adult should be sufficient for this process but two is recommended to ensure an adequate amount of gonads. If a second clam is desired, acquire a second clam from the container of live acclimated clams and follow steps 7-11

12. After flushing the clams free of gonads, dispose of the dead adults in an acceptable manner
    NOTE: A large number of transparent gonads should now be present in the bottom of the petri dish
13. Siphon up a proportion of the gonads from the petri dish using a sanitary plastic disposable pipette
14. Return to the container of 20-50 adult clams that you wish to induce
    NOTE: Make sure the adults in the container appear relaxed and are actively siphoning water.
15. Very discreetly lower the disposable pipette, which now contains gonads, into the container with siphoning adults
16. Release any air bubbles out of the pipette
17. Get as close to the incurrent siphon of each adult clam as possible without disrupting them.
18. Very slowly and softly squeeze the gonads in the pipette into the incurrent siphon (the larger of the two siphons) of the live adult clam
    NOTE: The gonads can be used on several adults in the container not just a single individual. You should be able to visually see the adult clams intake the gonads through their siphon and into their bodies
    NOTE: At times the adult may reject the gonads, by expelling water out of their incurrent siphon, just allow the clam to recover and continue to release gonads from the pipette into the incurrent siphon until you are comfortable that some gonads have been ingested
19. Continue this method until you have used almost all the gonads in the petri dish
20. For the final pipette, instead of releasing the gonads directly into the inhaling siphon of the adults, choose a corner of the container and place the pipette under the water almost parallel to the surface but pointed slightly downward (5% angle) and quickly release all the gonads into the container and allow them to disperse across the water column to the opposite side over the adult clams (mimicking a clam releasing veligers). If desired suck in a bit of water from the container and release the contents of the pipette again to clean out any remaining gonads from inside the pipette
21. Allow clams 2-3 days to begin releasing viable veligers
    NOTE: if clams do begin releasing veligers repeat the process or slightly increase temperature of room
22. During this time replace air stones
23. The release should be synchronous, with multiple adults releasing veligers at one time, however this is not always the case
24. Be careful with water exchange in the adult container during this time. It is ok to decant the surface water but care should go into not disturbing the benthos if veligers are being released
25. Siphon up veligers with a clean disposable pipette and place into a new 1.8 L container with de-chlorinated well water and supplemented aeration

26. Note the date that the veligers are removed from the adult container and placed into the new container.
27. A new container should be made every week so as not to over crowd previously collected veligers
28. Provide some organic material for the veligers to consume
    NOTE: After some time veligers may start to die off as a result of a naturally high mortality rate at that life stage.