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Effectiveness of EarthTec ® on killing invasive quagga mussels (Dreissena rostriformis bugenis) and preventing their colonization in the Western U.S.

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EFFECTIVENESS OF EARTHTEC® ON KILLING INVASIVE QUAGGA MUSSELS (*DREISSENA ROSTRIFORMIS BUGENSIS*) AND PREVENTING THEIR COLONIZATION IN THE WESTERN U.S.

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ABSTRACT

Effectiveness of EarthTec® on Killing Invasive Quagga Mussels (*Dreissena rostriformis bugensis*) and Preventing Their Colonization in the Western U.S.

by

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Dreissena rostriformis bugensis, an invasive species, also known as the quagga mussel, was discovered in Lake Mead, NV in January of 2007. In the four years since detection, quagga mussels have created tremendous economical, ecological, and human health impacts. The mussels clog pipes, ruin boat motors, and damage recreational equipment, and once established in the lake, routine maintenance is necessary to avoid further damage. Because of quagga mussels' high fecundity, planktonic veliger stage, and ability to attach to substrates with byssal threads, they have easily and swiftly spread to other lakes and reservoirs in the Lower Colorado River Basin. Several strategies have been employed to mitigate and control their spread. This thesis focuses on the most popular methods of chemical control. The first portion of the study evaluated the effectiveness of EarthTec®, a copper sulfate based biocide, on killing invasive quagga mussels (adults, juveniles, and veligers) in Lake Mead, NV-AZ, at six doses, 0, 1, 5, 10, 17, and 83 ppm. For adult mussels, 100% mortality was reached by 96 h treated with

17ppm and 83 ppm EarthTec®; by 168 h, more than 90% mortality was reached treated with 5 ppm and 10 ppm EarthTec®. For juvenile mussels, 100% mortality was reached by 48 h, 72 h, 72 h and 96h for groups treated with 83 ppm, 17 ppm, 10 ppm and 5 ppm EarthTec®, respectively. For veligers, an EarthTec® dose of 3 ppm or higher killed them within 30 min or less. The second portion of the study tested the effectiveness of EarthTec® on preventing veliger quagga mussel colonization from December 2010 to early February of 2011. Veligers were dosed with 0, 1, 2, and 3 ppm of EarthTec®. Control groups (0 ppm) had more colonized mussels than the groups treated with EarthTec® ($p > 0.01$). Statistical analysis showed that a dose of 2.6 ppm can prevent colonization of quagga mussels in Lake Mead under the experimental conditions. The results showed that EarthTec® is effective in killing adult, juvenile, and veliger quagga mussels and is effective against preventing veliger colonization. This study contributes to the understanding of chemical options that are available for quagga mussel control and prevention.

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CHAPTER 1

INTRODUCTION

National parks are the basis of conservation where the land is set aside for native animal habitats and human recreation and enjoyment. Lake Mead National Recreation Area in Nevada is diverse with different desert animal species, plants, and aquatic ecosystems. It is the largest reservoir by volume (3.5×10^{10} m^3) in the United States with four inflows, three basins, plus variable seasonal and annual operational patterns (LaBounty & Burns, 2005). In January 2007, the quagga mussel, an invasive species, was discovered in Lake Mead (LaBounty & Roefer, 2007). It has only been four years since the discovery of the quagga mussel, but they have left tremendous economic, ecological, and human health impacts.

The economic impact of zebra and quagga mussels in North America has been estimated at $1 billion/year (US Army Corps of Engineers, 2002). They invade and clog water intake pipes, water filtration, and electric generating plants. The mussels also ruin boat motors, damage recreational equipment, and once established in the lake, routine maintenance is necessary to avoid further damage. To prevent further damage and spread of quagga mussels, The Metropolitan Water District of Southern California plans to spend between $10-15 million per year (Wong & Gerstenberger, 2011). Quagga mussels alter the ecosystem by increasing water clarity and bioaccumulating contaminants. With their efficient filtering capabilities, quagga mussels remove suspended materials and nutrients from the water, making little or none available for native aquatic species that feed on the same nutrients (Claudi & Mackie, 1994). Both the zebra and quagga mussel are responsible for shifting the food web from a pelagic-based to a benthic-based one in

Lake Erie which can create a new pathway for contaminant transfer to top predators (Hogan, Marschall, Folt, & Stein, 2007). This same effect can occur in Lake Mead, and monitoring protocols are in place to assess the issue. Mercury levels have been measured in quagga mussels in Lake Mead, and the baseline research shows potential in using quagga mussels as a biomonitor of overall lake health (Mueting & Gerstenberger, 2010). Quagga mussels would make an effective monitor because of their ease in collection, sedentary lifestyle, and wide distribution.

Purpose of the Study

Because of quagga mussels' high fecundity, planktonic veliger stage, and ability to attach to substrates with byssal threads (Ram & McMahon, 1996), they have easily and swiftly spread to other lakes and reservoirs in the Lower Colorado River. Several strategies have been employed to mitigate and control their spread. This thesis focuses on the most popular method, chemical control. Using a copper sulfate based product, EarthTec®, this thesis examines its effectiveness on killing adult, juvenile, and veliger quagga mussels and also its effectiveness on preventing veliger colonization on fiberglass substrates.

Research Questions

- Can the US EPA-registered and NSF-certified algicide/bactericide, EarthTec®, be used as an effective molluscicide?
- What is the lowest concentration of EarthTec® that is effective in killing adult, juvenile, and veliger quagga mussels?

- What life stage of quagga mussel is most sensitive to EarthTec®?
- Is EarthTec® effective in preventing veliger quagga mussels from colonizing?
- What concentration of EarthTec® will be effective in preventing veligers from colonizing?

Hypotheses

- EarthTec® can kill adult, juvenile, and veliger quagga mussels at or below the US EPA's Safe Drinking Water Regulations at 1.3 ppm of copper
- The effective dose of EarthTec® in killing quagga mussels will be dependent on the stage of the quagga mussel's lifecycle, where veligers will be more sensitive to EarthTec® than adults
- EarthTec® will be effective below 3 ppm (0.18 ppm of Cu^{2+}) in preventing veliger quagga mussel colonization
- The dose to prevent veliger colonization will be below the US EPA's Drinking Water Regulations for copper at 1.3 ppm in an experimental situation

CHAPTER 2

LITERATURE REVIEW

Zebra and Quagga Mussel Biology

Spread of Dreissenid Mussels

The Russian naturalist, Peter Pallas, was the first to discover zebra mussels (*Dreissena polymorpha*) in the Ural River in 1769 (Ludyanskiy, McDonald, & MacNeil, 1993). The zebra mussel quickly spread across Western Europe, travelling through rivers and tributaries, and finally entering North America via ballast tanks on large ships (Carlton, 1993). The zebra mussel was first documented in North America in 1988 in Lake St. Clair (Hebert, Muncaster, & Mackie, 1989). However, there is new evidence that zebra mussels were detected in Lake Erie on natural gas wellheads and well markers in 1986. Less than a year later, zebra mussels were found off the shore of a water treatment plant and in vessel fouling on Lake Erie. As the population increased in 1988, it is hypothesized that the zebra mussels then spread to Lake St. Clair (Carlton, 2008). Once established, they spread quickly to all the Great Lakes and then entered eight river systems such as the St, Lawrence, Hudson, Mississippi, Ohio, Illinois, Tennessee, Susquehanna, and Arkansas (Ludyanskiy et al., 1993) (Figure 1). The first occurrence of the quagga mussel in North America was documented in 1989 in Lake Erie (Mills, Dermott, Roseman, Dustin, Conn, & Spidle, 1993). This morphologically and genetically different species of *Dreissena* was identified as a different species and then given the name quagga mussel in 1991 (May & Marsden, 1992). *Dreissena rostriformis bugensis* is indigenous to the Dnieper River drainage of Ukraine and Ponto-Caspian Sea. The mussels were discovered in the Bug River by Andrusov, who named the species in

1897 after the “quagga”, an extinct African relative to the zebra (Mills, Rosenberg, Spidle, Ludyankiy, Pligin, & May, 1996; May & Marsden, 1992). Once in the United States, the quagga mussel eventually moved towards the southwest region (Figure 2). Quagga mussels were discovered in Boulder Basin of Lake Mead, NV on January 7, 2007, and that same year, mussels were confirmed in Lake Mojave, NV and Lake Havasu, AZ (LaBounty & Roefer, 2007).

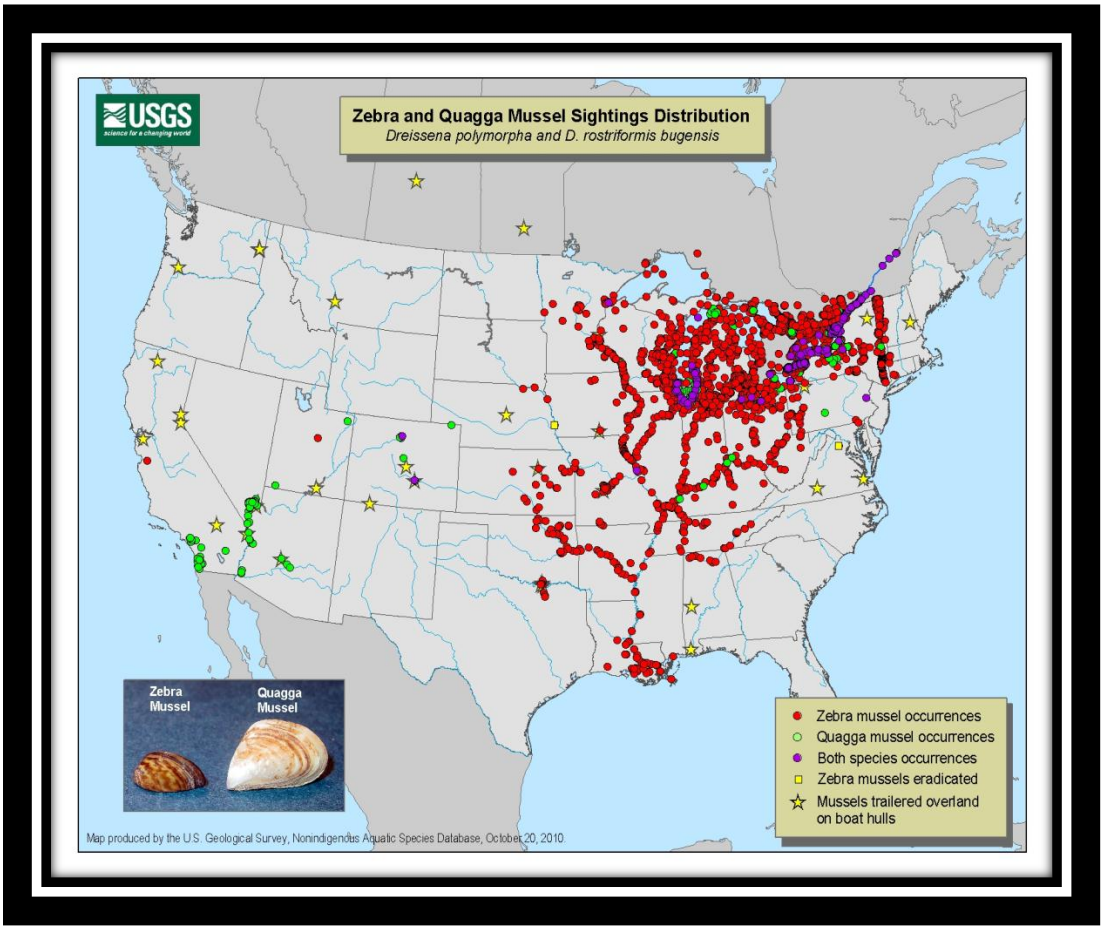

Figure 1 Zebra and Quagga mussel distribution in the US October 2010 Image from US Geological Survey (USGS), Nonindigenous Species Database. Retrieved [05 Feb 2011] from http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/maps/current_zm_quag_map.jpg

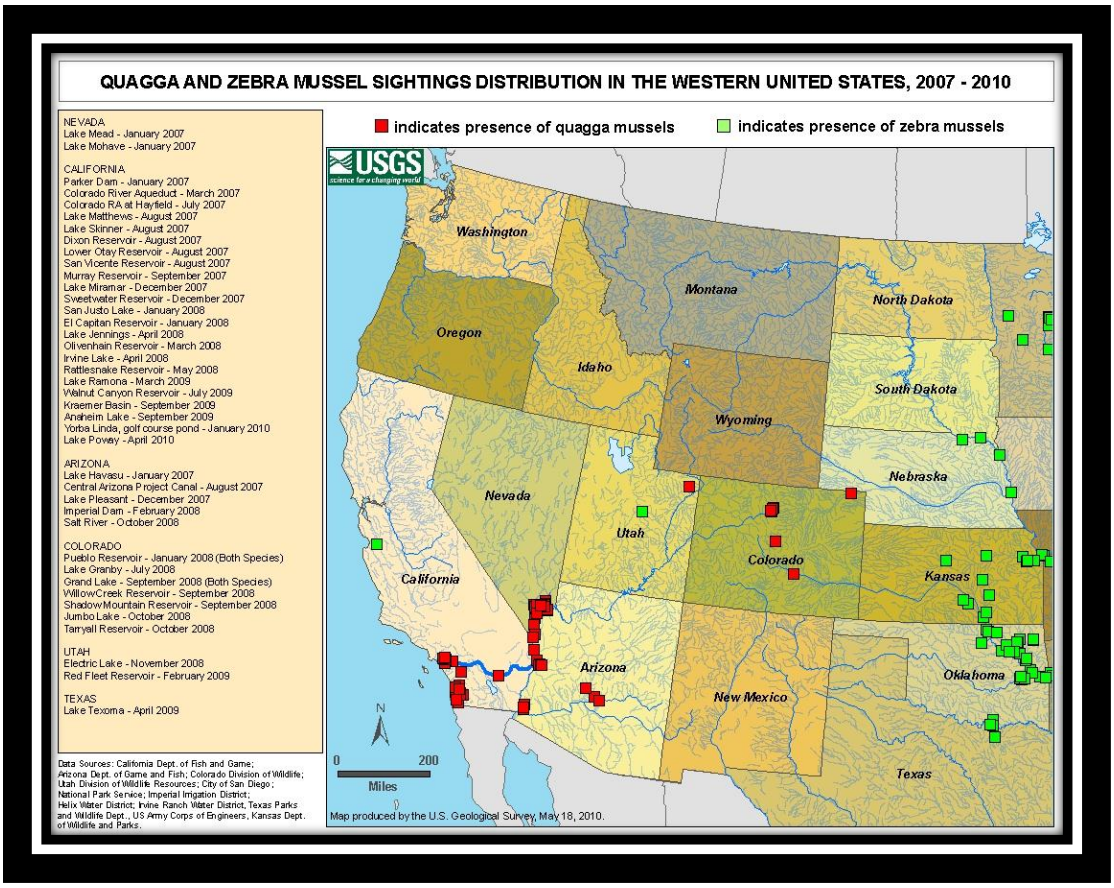

Figure 2 Zebra and quagga mussel distribution in Western US May 2010 Image from USGS. Retrieved [05 Feb 2011] from http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/maps/southwest_quagga.pdf

Morphological Differences

The two species of zebra and quagga mussels can be visually differentiated via their shells. *D. polymorpha* derives its name from the zebra-like pattern on its shell, and "polymorpha" refers to the many color patterns on the shell (Claudi & Mackie, 1994). Zebra mussels have a flat ventral margin which allows the mussel to remain upright when placed on a flat surface (Mills et al. 1996). *D. bugensis* shells are less flattened on the bottom and are rounder, whereas the zebra mussel is more triangular (Figure 3) (Marsden

et al., 1996). Both zebra and quagga mussels have permanent openings in which the byssal apparatus extends, and in the quagga mussel, this is more anterior (Claudi & Mackie, 1994). The best way to identify dreissenid species is to analyze the DNA sequence through allozyme electrophoresis (May & Marsden, 1992; Spidle, Marsden, & May, 1994). The internal morphology of zebra and quagga mussels does not differ. Ciliary action is used to move water in the body cavity via the inhalant siphon. Digestible food particles move towards the mouth, and unpalatable particles are bound in mucous and then rejected via the inhalant siphon as pseudofeces (Crosier & Molloy, 2001). On the ventral side of the shell, the mussel has a muscular foot that is used for moving on a substrate and secretion of byssal threads (Claudi & Mackie, 1994).

Figure 3 Morphological differences between *Dreissena polymorpha* and *Dreissena rostriformis bugensis* Source: Crosier & Molloy, 2001)

Life Cycle and Reproduction

Both zebra and quagga mussels have a high rate of fecundity (Ram & McMahon, 1996). Adult mussels are sexually mature, reach a length of 4 cm, and typically live three to five years (Marsden, 1992; Nichols & Black, 1994). Life of a mussel begins with external fertilization of an egg by sperm in the water column (Crosier & Molloy, 2001). Water temperatures are usually between 12°C and 15°C for zebra mussel egg and sperm to be seen (Claudi & Mackie, 1994), but quagga mussels have been seen to spawn at a low temperature of 4.8°C (Roe & MacIsaac, 1997). Two days after fertilization, a trochophore (57-121 µm) develops (Nichols & Black, 1993). This is a rather short phase, and the trochophore metamorphoses into a veliger. A veliger (150-250 µm) is described as a ciliated, free-swimming planktonic stage which can be transported in water currents (Marsden, 1992). Within two to nine days after fertilization, the veliger begins to secrete a D-shaped or straight hinged shell, which is still transparent (Crosier & Molloy, 2001). Within nine days, the shell has a more pronounced umbonal region near the hinges and is more rounded. The umbonal stage represents the last stage in which a veliger will be free swimming and found in the plankton (Marsden 1992; Claudi & Mackie, 1994). As the veliger in the umbonal stage grows, the velum (the organelle which facilitates in swimming) develops into a siphon, the foot lengthens, organ systems begin to develop, and the organism is now a pediveliger (200-300 µm) (Claudi & Mackie, 1994; Martel, 1993). Pediveligers are too heavy to be carried with the water current, so they fall onto substrates and then have the ability to crawl (3.8 cm/hr) around before extruding byssal threads, which allows them to attach to a substrate and become a plantigrade (> 500 µm) (Lewandowski, 1982; Marsden, 1992). This is the last phase before the veliger becomes a juvenile, and begins feeding with gills instead of a velum, and moves solely with its

foot (Crosier & Molloy, 2001). In the juvenile stage, the mussel has a more triangular or mussel-like shape, grows about 83-200 µm/week (Hincks & Mackie, 1997), and becomes sexually mature around 7 mm (Marsden, 1991). It has been shown that quagga mussels grow at a faster rate than do zebra mussels. Given high food levels, quagga mussel growth was three times greater than zebra mussel growth at high temperature, and as high as 19 times greater than zebra mussel growth at a low temperature (Baldwin et al., 2002).

Settlement

Veligers will live in the water column for weeks before falling out and settling on a hard substrate, becoming a juvenile, and eventually forming a colony (Martel, 1993). Successful mussel colonies have been found on anything natural from rock, benthic sediment, to man-made substrates such as boats, docks, buoys, and trash.

To mitigate and control colonization of quagga mussels, it is important to understand the mechanism behind settlement. The shell shape of zebra and quagga mussels is advantageous in that the flat, ventral surface allows the animal to be pulled tightly against the substrate by the byssal threads, which aids in the protection from predators. The umbone is adjacent to the substrate which gives the mussel upright stability at the surface of the substrate and the shell is tapered dorsally which makes it difficult for predators to pry the shell from the substrate (Claudi & Mackie, 1994). Zebra mussels occur in large numbers at all depths of the epilimnion (3-7 m) but have been found as deep at 15 m (Claudi & Mackie, 1994). Zebra mussels have been found in the hypolimnion; however, the cold waters limit their growth and reproduction (Claudi & Mackie, 1994). In Lake Mead, quagga mussels have been found over 108 m deep (Wong & Moore, unpublished data). It has been shown that quagga mussels settle at a larger size than zebra mussels.

Martel et al, (2001) explain that the mean size in settlement between the two species could be explained by a longer planktonic development time, ability to delay settlement, or a faster larval growth rate in the quagga mussel.

Numerous studies have been conducted to determine the type of substrate that quagga mussels prefer and which type will deter their settlement (Martel, Mathieu, Findlay, Nepszy, & Leach, 1994; Wainman, Hincks, Kaushik, & Mackie, 1996; Marsden & Lansky, 2000; Aquatic Environmental Consulting, 2008). When settling on plates, mussels prefer stainless steel, polypropylene, black steel, pressure treated wood, Teflon, polyvinyl chloride (PVC), aluminum, and galvanized steel (Kilgour & Mackie, 1993). Based on the toxicity of copper, brass, and galvanized iron, mussels tend to not settle on these metals, and if they do, it usually takes longer to be colonized versus other metals (Kilgour & Mackie, 1993). Suitable substrates for mussels are based on texture, chemical composition, orientation in the water, and presence of light and a biofilm (Kavouras & Macki, 2003). Marsden & Lansky (2000) found that zebra mussels prefer upper, horizontal surfaces versus lower surfaces, textured versus smooth surfaces, shaded versus sunlit surfaces, and plastics versus glass. Quagga mussels will settle on hard substrates, but unlike zebra mussels, they will also colonize deeper waters and on softer substrates (i.e., sediment surfaces) (Mills et al., 1993). Under acceptable conditions, 99% of veligers do not reach a suitable substrate and attach (Aquatic Environmental Consulting, 2008). Daily settlement rates are strongly correlated with the concentration of veligers found in the water column (Martel et al., 1994). Understanding zebra and quagga mussel substrate preference can aid in controlling for the invasive species in vulnerable areas.

Control Methods for Zebra and Quagga Mussels

Measures to control zebra and quagga mussels include mechanical, thermal, desiccation, biological, and chemical protocols. These methods have been used alone, or by combining methods. While all methods are important, a combination of methods may be the best and safest way to completely eradicate zebra and quagga mussels.

Mechanical cleaning involves using mechanical scrubbers to remove mussels from all external structures and large diameter piping (Claudi & Mackie, 1994). This may not be the ideal method because the pipeline is unavailable during cleaning and the structures may not be able to withstand the pressure generated by the scrubbers. Methods using hot water spray have been proven effective against zebra and quagga mussels within minutes (Morse, 2009; Comeau et al., 2011). Power plants or industries where excess heat is available to raise water temperatures have the advantage to combine chemical and heat control strategies. For example, the addition of chlorine at elevated temperatures can reduce mortality times of zebra mussels by as much as three orders of magnitude compared to oxidant addition at ambient temperatures (Harrington, Van Benschoten, Jensen, Lewis, & Neuhauser, 1997). Zebra and quagga mussels can live up to 30 days out of the water depending on ambient temperature and humidity (McMahon, Ussery, & Clarke, 1993). Using desiccation as a mechanism for zebra and quagga mussel control can be time consuming and costly. The entire facility would need to be shutdown to drain the pipes and allow the mussels to dry out (Claudi & Mackie, 1994). It would be advantageous to use hot air to heat the pipes to speed up the process.

The majority of this thesis will focus on chemical control of zebra and quagga mussels. There are different ways of beginning a chemical control protocol. It can be

accomplished by using molluscicides to kill mussels or develop molluscistatic products which do not kill the mussels, rather deter attachment. Chemicals that are irritating enough to deter attachment may be useful in preventing a secondary infestation once the primary infestation has been removed (Fisher & Bernard, 1991).

Zebra and Quagga Mussel Chemical Control

There are many methods that may be effective in controlling zebra and quagga mussels. The most common and widely used method in both the United States and Europe is chemical control (Claudi & Mackie, 1994). Following the introduction of the nonindigenous zebra and quagga mussels, a number of chemicals with unknown and known molluscicidal properties have been proposed for use in controlling invasive mollusks (Sprecher & Getsinger, 2000). No matter what chemical, it must be cost effective, not be harmful to the surrounding aquatic ecosystem, and safe as an additive in drinking water.

The way in which a chemical is used is as important as what chemical is chosen. Application strategies are also important to follow when administering a chemical or toxicant. There are five basic ways to apply a chemical treatment: end of season, periodically, intermittently, semi-continuously, and continuously (Claudi & Mackie, 1994; Sprecher & Getsinger, 2000). The end of season treatment is applied at the end of the breeding season to kill adult mussels that are established within the water system (Sprecher & Getsinger, 2000). As a result of this method of application only being applied once a year, dead mussel debris can build up and this may be a problem to water treatment facilities. Periodic treatment is similar to end of the year treatment, but is done more frequently. While adult mussels are still the target, periodic treatment may also be

effective in preventing new settlement of juveniles if administered frequently enough (Claudi & Mackie, 1994; Sprecher & Getsinger, 2000). Intermittent, semi-continuous and continuous treatments are all designed to prevent new settlement of mussels in raw water systems. Oxidizing chemicals work best with intermittent treatments at frequent intervals (i.e., every 6, 12, and 24 h) (Claudi & Mackie, 1994). The aim is to destroy post-veliger stages of development to prevent further mussel infestation. Semi-continuous treatment creates a constant state of stress in mussels. The treatment schedule can be adjusted to 15 min on and then 45 min off, effectively controlling all stages of mussels in the piping systems. Continuous treatment is used when a low concentration of a chemical can be used continuously. It is typically used in systems that cannot tolerate any biofouling, such as fire protection systems (Claudi & Mackie, 1994). Chemical control of mussels has proven to be very effective in closed systems, where higher dosages required for adult eradication restricts the use of the chemical chosen based on field management plans (Kennedy, Millward, Steevens, Lynn, & Perry, 2006). When considering the best option for chemical control of quagga mussels, the most chemically sensitive life stage should be identified and the method of treatment tailored accordingly.

Oxidizing Chemicals

Chlorination

The most popular and least expensive chemical used for control of invasive mussels is chlorination, where chlorine is added as chlorine gas or as liquid sodium hypochlorite (Claudi & Mackie, 1994; Rajagopal et al., 1996; Sprecher & Getsinger, 2000). The benefits of chlorine are that it is effective at low concentrations and efficient against all fouling categories ranging from bacteria to mollusks. It not only kills adult quagga

mussels, but is effective in preventing embryonic forms (i.e., veligers) from settling in raw water piping systems increasing the water facility's efficiency (Jenner and Janssen-Mommen, 1993).

Factors that influence the effectiveness of chlorine for quagga mussel control include temperature, physical state of the mussel, and water quality. It has been shown that higher temperatures facilitate increased uptake of chlorine compounds that increase its toxic effects (Zolotareva, Makhonina, & Dyga, 1978). The age, size, and developmental stage of the quagga mussels also have an effect on the quantity of chlorine required to achieve 100% mortality. The expectation is that adult and juvenile mussels are less resistant to the toxicant compared to veliger stages. Treatment at the most vulnerable stages of development would not require high levels of chlorine, thus keeping cost low (Claudi & Mackie, 1994). Following reproduction, mussels are physically exhausted, so implementing a chlorine regimen at this time would reduce the amount of chemical and length of time it would otherwise take if the protocol was delivered at a different time of the year (Bayne et al., 1976). The decomposition rate of chlorine residual through oxidation-reduction reactions and volatilization also affects the toxicity of chlorine. The rate that residuals are lost is influenced by the type and amount of organic and inorganic compounds that are present (Claudi & Mackie, 1994). For instance, if reducing agents are present in the water, the decomposition rate of chlorine residuals increases, resulting in reduced mussel mortality due to the lessening of the chlorine toxicity.

Chlorine controls mussels through an oxidation process either directly on the adults or through inhibition of settlement and growth of the veligers. Hypochlorite compounds react with water to form hypochlorous acid (HClO), which will then dissociate to

hydrogen ions (H^+) and hypochlorite (ClO^-) (Sprecher & Getsinger, 2000). Undissociated HClO is a strong oxidizing agent that can damage membranes by diffusing through the cell wall and disrupt enzyme activities (Claudi & Mackie, 1994). Mussels are able to sense chlorine in low doses when it is present in the water. Mussels will react by closing their valves, and stop filter feeding, making it necessary to survive off stored food reserves and anaerobic respiration (Rajagopal et al., 1997; Rajagopal et al., 2002). Since mussels try to avoid the chemical, they may actually die from asphyxiation or limited glycolysis over a prolonged period (Van Benschoten, Jensen, Harrington, & DeGirolama, 1995).

Trihalomethanes (THM) are formed as a by-product of chlorination when it is used to disinfect drinking water. These are formed when chlorine reacts with organic or inorganic material already present in the water being treated. THM are halogenated single carbon compounds that include chloroform, bromodichloromethane, dibromochloromethane, and bromoform. THM are linked to adverse health effects and may even be carcinogenic to animals (Cotruvo & Regelski, 1989). The US Environmental Protection Agency (US EPA) has set a standard for the maximum allowable annual average concentration level of total THMs of 80 ppb (US EPA, 2010). In cases where THM exceeds the US EPA's limit, an alternate form of chemical control should be implemented.

Like all control methods, the use of chlorine to kill adult and juvenile quagga mussels and prevent veliger quagga mussels from settling, has advantages and disadvantages. The advantages include proven efficacy in removing mussels from most raw water systems, lethality at low concentrations, no bioaccumulatory properties, ease in

measurement of total residue oxidant (TRO), relatively low cost, and chlorination systems being simple to construct and maintain. Disadvantages include safety problems related to transport and storage of liquefied chlorine, and problems maintaining the allowable limit of THM by-products at points of discharge due to variations in chlorine demand (Claudi & Mackie, 1994).

Chlorine Dioxide

Chlorine dioxide may be useful as an alternative to chlorine if THMs become a serious problem (Sprecher & Getsinger, 2000). This chemical is a biotoxic oxidant that causes damage to membranes in the target organism. Chlorine dioxide has advantages of being efficacious at low concentrations, does not produce THM, and only requires short treatment duration. The disadvantages of chlorine dioxide include on-site generating equipment being required, difficulties in the storage of sodium hypochlorite and hypochloric acid (precursor chemicals), and the conversion of the dioxide to chlorite, which limits the amount of chlorine dioxide that can be applied without excessive chlorite discharge (Sprecher & Getsinger, 2000).

Chloramine

Chloramine is a family of organic compounds with the formulas R_2NCl and $RNCl_2$ that may be a suitable alternative to chlorine when THM concentrations become too high. Chloramines are formed naturally when free available chlorine reacts with nitrogen compounds, such as ammonia and amino acids. Low doses of chloramine compounds result in a high rate of veliger mortality in both static and flow-through tests (Van Benschoten et al., 1993).

Non-chlorine Oxidizing Chemicals

In addition to chlorine and chlorine dioxide, the oxidizers bromine and potassium permanganate are also effective in controlling quagga mussels.

Bromine

Bromine can be used in different forms including activated bromine, sodium bromide, bromine chloride, or a mixture of bromine and chlorine. When water pH is above 8.0, bromine is a more effective oxidizing agent (Fellers, Flock, & Conley, 1988). Bromine exerts its lethality to quagga mussels by destroying vital tissues and produces more rapid effects in veligers compared to adult mussels. It is recommended that bromine compounds be applied three times a year, subsequent to reproduction peaks (Sprecher & Getsinger, 2000). Bromine is less effective in preventing colonization of veligers compared to chlorine and bromine does not seem to hinder growth rates of mussels that do colonize (Bidwell, Cherry, Farris, Petrille, & Lyons, 1999).

Potassium Permanganate

Potassium permanganate is an oxidizer used in municipal water treatment facilities for purification. It is effective in controlling adult mussels and inhibits veliger settlement (San Giacomo & Wymer, 1997). Potassium permanganate is best used in the summer season during the veliger settlement phase. Adult mussels retract their siphons while potassium permanganate diffuses through the water, and the mussels may die through asphyxiation or starvation (Sprecher & Getsinger, 2000). A drawback to using potassium permanganate is that it can produce a pink or yellow color in treated water (San Giacomo & Wymer, 1997). Other considerations when deciding whether to use potassium permanganate is cost associated to the amount of chemical needed, the solubility of

potassium permanganate in the water, and the size of the delivery system that is required (Claudi & Mackie, 1994).

Non-oxidizing Chemicals

Non-oxidizing chemicals that were developed for bacterial disinfection and algae control have also been tested as molluscicides (Claudi & Mackie, 1994). These are generally more potent, more easily and safely handled and more easily applied to raw water systems than chlorine; however, there is a higher cost associated with non-oxidizing molluscicides compared to chlorination strategies (McMahon, Shipman, & Long, 1993). Non-oxidizing molluscicides are generally used in closed-loop systems due to concern over their persistence in the environment.

Quaternary and Polyquaternary Ammonium Compounds

Polyquaternary ammonium molluscicide, poly[oxyethylene(dimethyliminio)ethylene(dimethyliminio)ethylenedichloride], also known as "polyquat" induces mortality in mussels when it is given as a semi-continuous dose. This dosing strategy not only reduces the amount of discharge, but cost in using the chemical is also reduced. The compound binds to negatively charged surfaces on the mollusk membrane. The mussel does not detect the polyquat; therefore, the organism does not close its valves and quickly dies (Sprecher & Getsinger, 2000). Polyquat compounds are also effective in rapidly killing quagga mussel veligers (Britton & Dingman, 2011), but in low doses, does not completely prevent colonization (Martin, Mackie, & Baker, 1993).

Potassium

In closed loop-systems, potassium compounds are effective and economical in controlling for quagga mussels. While nontoxic to larger organisms, such as fish, they are toxic to bivalve organisms at low concentrations (Claudi & Mackie, 1994; Waller et al., 1993). The toxicity to non-target organisms is an environmental risk and that is why potassium compounds would not be suitable for once-through systems. Potassium ions are toxic to mussels in that the ions interfere with membrane integrity and respiration (Waller et al., 1993). Fisher et al., (1991) hypothesizes that potassium kills adult mussels by destroying the membrane integrity of the gill epithelium, making it impossible for the mussel to respire.

Copper

Natural metallic copper was discovered and used by some of the oldest civilizations on record. Copper compounds have been used in medicine and agriculture for centuries. For example, verdigris (basic copper acetate) was used as a pesticide in Roman vineyards, Bordeaux mixture (cupric hydroxycarbonate) was used to combat mildew on grapes, and the "bluestone" (cupric sulfate pentahydrate) is used as an algicide worldwide (Crosby, 1998).

Historically, copper has not been used as a means of preventing nor killing zebra and quagga mussels in municipal settings; copper has been added to antifouling coatings for ships to prevent barnacle growth (Claudi & Mackie, 1994) and as an antifouling agent on underwater pipes (Dormon et al., 1996). The general toxicity of copper has been found to be successful; however, the copper ions leach from the coatings and result in unacceptable copper concentrations in water systems. Currently, the US EPA Drinking

Water Regulations for copper is a maximum containment level (MCL) of 1.3 mg L^{-1} or 1,300 ppb (US EPA, 1991). This is a level of contaminant below which there is no known or expected health risk to humans or animals.

Because of the environmental side effects that occur with copper toxicity, it is important to know which stage of the mussel's life cycle will be the most vulnerable. It has been suggested that the larvae may be the best target for copper control using periodic or continuous chemical treatment to reduce population spread from one water body to another (Waller et al., 1993). Targeting larvae may also reduce the amount of chemical that is needed per treatment. Mussel larvae are significantly smaller and more exposed to their environment because they do not have a protective shell. Chemical management strategies targeting early larval stages of zebra and quagga mussels are likely more cost efficient and less prone to non-target environmental impact compared to strategies aimed at adults (Kennedy et al., 2006).

EarthTec®

EarthTec® is formulated by blending copper sulfate pentahydrate with Earth Science Laboratory's base acid, "ET-3000". EarthTec® is registered with the US EPA (registration No. 64962-1) as an algicide/bactericide and certified to American National Standards Institute (ANSI) and National Sanitation Foundation (NSF) Standard 60 as a drinking water additive. It is used in lakes, ponds, reservoirs, municipal drinking and wastewater systems, irrigation canals, animal confinement pits, treatment lagoons, and other water systems. The biologically active ingredient in EarthTec® is the cupric ion form of copper (Cu^{2+}). The cupric ion typically remains unattached to inorganic elements

in most waters that are low in pH and hardness, which allows the ability of copper to exert toxic effects to microorganisms.

Cu^{2+} reacts with organic molecules that form living tissues. This active form makes Cu^{2+} available to be bound, or chelated by organic molecules. The Cu^{2+} in EarthTec® exerts toxic effects in microorganisms (i.e., bacteria or algae) by first affecting the permeability of the cell membrane, causing a loss of potassium ions, and then Cu^{2+} accumulates in cell walls, membranes, and organelles which contain negatively charged molecules (i.e. S-H and COOH groups) (Earth Science Laboratories, 2010). The cupric ion is chelated by the negatively charged components in the cell walls. At this point, copper moves via non-metabolic transport directly to the chloroplast of the cell. The cupric ion becomes chelated by the compounds in the chloroplast membranes, and photosynthesis is inhibited and irreparable, causing death in the microorganism. Once the microorganism has died, copper remains chelated to the negatively charged components of the cell walls, which degrade over time, and the copper chelates will settle to the bottom of the water column (Earth Science Laboratories, 2010). The copper is no longer biologically active, nor capable of exerting toxic effects to other organisms.

EarthTec® contains a proprietary copper "carrier" that holds copper (i.e. Cu^{2+}) in solution over long periods in a wide range of water conditions. This "carrier" is what sets EarthTec® apart from other copper compound chemicals on the market (Earth Science Laboratories, 2010). With high pH and calcium levels, the effectiveness of copper sulfate as an algicide is limited because cupric ions will recombine into less soluble forms which will precipitate out of the water column (McKnight, Chisholm, & Harleman, 1983). Large quantities of copper sulfate would need to be applied in order to achieve maximum

control of algae growth (Hanson & Stefan, 1984). It would not be possible to control the amount of copper sulfate that is released into the aquatic environment and hazardous accumulation may occur. Cu^{2+} in EarthTec® is free to act upon algae and bacteria while still being held in the solution and the "carrier" prevents the cupric ion from chelating with other organic molecules that are found in the water column (Pasek, 1993). In other words, when using EarthTec®, a controlled amount of copper is released into the water limiting undesired effects. EarthTec® will rapidly and evenly self-disperse throughout the body of water to which it is applied. Mixing or agitation is not necessary which would minimize equipment and labor costs. Regardless of dilution rate, EarthTec®'s active ingredient will always be uniformly distributed (Earth Science Laboratories, 2010).

EarthTec® has been endorsed from various water agencies such as: Enviro-Reps International, Inc., Stone Harbor Golf Club, The James Group (Fountain Service), City of Newberry in South Carolina, City of Jefferson, Douglas County Water and Sewer Authority, Weber Basin Conservancy District, Utah Murray Reservoir, and Water System in Chorrera (Republic of Panama).

CHAPTER 3

METHODOLOGY

This thesis is based on two separate experiments. One, testing the effectiveness of EarthTec® on killing adult, juvenile, and veliger quagga mussels, and the other experiment examines the use of EarthTec® in preventing veliger colonization.

The Effectiveness of EarthTec® on Killing Quagga Mussels

Collection of Data

Specimen Collection

Adult, juvenile, and veliger specimens of *D. rostriformis bugensis* were collected from Lake Mead, NV (36°1'50.69"N; 114°46'12.95"W). Adults and juveniles were collected from rope substrates between 10 and 20 m, and veligers were collected off the dock at 30 m. A National Park Service permit was obtained, granting permission to collect quagga mussels (APPENDIX 1). Immediately following collection, the samples were brought back to the Nevada Department of Wildlife's (NDOW) hatchery in Boulder City, NV to acclimate for five days, in ten gallon tanks. The aquaria used for acclimation was stocked exclusively with water pumped directly from Lake Mead and equipped with a flow through system and aerated stones for the mussels. The specimens were then divided and placed into 24 fine media mesh bags with 12-15 mussels per bag. Mussels greater than 11 mm were considered adults; mussels less than 11 mm were considered juveniles. Veliger quagga mussels were collected from Lake Mead, NV for four mornings using a 64 µm pore size plankton net. The net was lowered to 30 m because a high abundance of veligers are found at that depth (Mueting, 2007). Approximately 15

vertical tows were used to collect veligers each day, with each ml of sample containing 3 – 30 veligers. Samples were brought back to NDOW's hatchery, and divided into small glass petri dishes (VWR Glass Petri Dish, 60 x 15 mm).

Dosing and Working Solution

Six concentrations of EarthTec® solution were used for the adult, juvenile and veliger toxicity tests: control (0), 1, 5, 10, 17, and 83 ppm with corresponding Cu^{2+} concentrations of control (0), 0.06, 0.3, 0.6, 1, and 5 ppm, respectively (Table 1). One gallon of EarthTec® per 1,000,000 gallons of water yields a rate of 0.06 ppm of metallic copper which equals 1 ppm of the target concentration of EarthTec®. Before beginning each experiment, working solutions of 10,000 and 1,000 ppm were prepared. 100 ml of Lake Mead water was filtered (Whatman Grade 54, low ash filter paper) and added to a beaker. One ml of EarthTec® was added to a clean 100 ml volumetric flask. A 10,000 ppm working solution is created when 99 ml of filtered water is added to the volumetric flask with the one ml of EarthTec®. Likewise, a 1,000 ppm solution is created when 99.9 ml of filtered Lake Mead water is added to another clean, volumetric flask with 0.1 ml of EarthTec®. Table 1 outlines the amount of working solution and the corresponding target ppm for tanks containing quagga mussel adults and juveniles. Table 2 outlines the amount of working solution and the corresponding target ppm for quagga mussel veligers. An additional dose of 3 ppm (0.18 ppm of Cu^{2+}) was added to the veliger experiment.

Table 1 Toxicity test of different concentrations of EarthTec® on quagga mussel adults or juveniles

Cu2+ (ppm)	EarthTec® (ppm)	Volume needed of working solution (ml)	Dilution (ppm)
0	0	0	0
0.06	1	0.1	1,000
0.3	5	0.5	1,000
0.6	10	1	1,000
1	17	0.17	10,000
5	83	0.83	10,000

Table 2 Toxicity test of different concentrations of EarthTec® (diluted 1,000 ppm) on quagga mussel veligers

Cu^{2+} (ppm)	EarthTec® (ppm)	Volume needed of working solution (ml)
0	0	0
0.06	1	0.01
0.18	3	0.03
0.3	5	0.05
0.6	10	0.1
1	17	0.17
5	83	0.83

Adult and Juvenile Toxicity Tests

Toxicity tests were conducted at the NDOW's hatchery at Lake Mead, NV in a temperature controlled room. Only healthy mussels, in the fine media mesh bags, were used for experimentation. The duration of both the adult and juvenile portions of the toxicity tests was a total of 7 days (168 h). Four replicates of the six treatment groups (including controls) were used for the toxicity tests. In total, for each adult and juvenile

test, 240 mussels of roughly equal size (~11 mm – 23 mm) were used for the toxicity experiments (10 mussels × 6 treatment groups × 4 replicates = 240 total mussels). Each replicate was placed in a mesh bag and immersed in a beaker with raw Lake Mead water and the appropriate dose of EarthTec® (total volume equals one liter). Each beaker was aerated with an air stone and kept in a 22°C water bath to mimic the epilimnion water temperature of Lake Mead. Each group was fed 0.1 ml of *Isochrysis sp* (1×10^6 cells/ml) daily.

Veliger Toxicity Tests

For the veliger toxicity portion of the experiment, the Ecological Effects Test Guidelines for bivalve acute toxicity test was followed as outlined by the US EPA. Unlike the adult and juvenile tests, this portion of the experiment did not exceed 48 h (US EPA, 1996). From the collected samples, 2 ml of veligers, the working solution of EarthTec®, and filtered Lake Mead water (total volume equals 10 ml) was pipetted into small, glass petri dishes (VWR Glass Petri Dish, 60 ×15 mm) and examined under a stereo microscope (Olympus Stereo Zoom, model SZ4045ESD) to assess viability. Both dead and alive veligers were counted and documented for each petri dish.

Adult, Juvenile, and Veliger Mortality

Mortality was checked every 24 h from the beginning of the experiments for adults, and mortality was checked at 6 h and 12 h followed by every 24 h for the juveniles. To test for mortality, gaping mussels were gently prodded on their shell valves, and individual mussels that do not respond by immediate shell closure were stimulated in the area of their siphons. Mussels that still did not respond to siphon stimulation, had their shell valves forcible closed with forceps. The mussel was considered dead if it

immediately reopened upon release of the forceps (Harrington et al. 1997; Morse, 2009; Comeau et al., 2011). Dead mussels were removed, measured, recorded, and placed into a different, labeled mesh bag. They were then transferred to a flow through system and mortality was confirmed 24 h later. The experiments for both adult and juveniles lasted 7 days (168 h) each (Table 3). Mussels that were alive at 168 h were also measured and recorded.

Table 3 Experimental design of toxicity test of EarthTec® on quagga mussel adults and juveniles

Mussels	Cu^{2+} (ppm)	EarthTec® (ppm)	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mortality assessment time
Adults	0	0	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	0.06	1	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	0.3	5	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	0.6	10	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	1	17	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	5	83	10	10	10	10	24 h, 48 h, 96 h, 120 h, 144 h, 168 h
Juveniles	0	0	10	10	10	10	6 h, 12 h, 24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	0.06	1	10	10	10	10	6 h, 12 h, 24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	0.3	5	10	10	10	10	6 h, 12 h, 24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	0.6	10	10	10	10	10	6 h, 12 h, 24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	1	17	10	10	10	10	6 h, 12 h, 24 h, 48 h, 96 h, 120 h, 144 h, 168 h
	5	83	10	10	10	10	6 h, 12 h, 24 h, 48 h, 96 h, 120 h, 144 h, 168 h

Veligers that exhibited ciliary movement during a two-minute observation period (Britton & Dingman, 2011), or if internal organs were moving, they were counted as alive. After the veligers were counted, the EarthTec® dilution was added with a light swirl to the petri dish, followed immediately by a determination of mortality using cross-polarized light (CPL) microscopy. Veligers are birefringent due to the crystalline structure of the calcite in the larval shell; hence, they stand out against a dark background. Because of the concentric arrangement of the crystals within the shell, the portions of the shell in line with the axes of the filters are not birefringent thus making the shells appear as glowing crosses (Johnson, 1995). Using CPL microscopy allowed for a higher degree of specificity when assessing mortality of veligers. When veligers stop moving, or internal organs appear to cease, mortality was assessed. If 100% mortality is not observed within 3 h, the petri dish was set aside and examined every 12 h thereafter, until 36 h was reached. Between each dose, the controls were examined to ensure viability.

The Use of EarthTec® in Preventing Veliger Colonization

Experimental Design

The colonization experiment was performed in two phases. The first phase was one month long, with six controls and six treatments of 1 ppm of EarthTec®. The second phase occurred when the first phase was completed with four controls, four treatment groups of 2 ppm, and four treatment groups of 3 ppm. Twelve 10-gallon tanks equipped with air stones were used. Prior to experimentation, each tank was filled with 25 L of raw Lake Mead water using a one L beaker. Each tank had fiberglass substrates (79 × 68

× 1.66 mm) hung with Trilene fish wire from the shelf above. Five days before the experiment, the fiberglass substrates soaked in Lake Mead water to form a biofilm. The substrates were used to measure colonization of veligers. Each week, half the water in the tanks was exchanged and replaced with fresh, raw Lake Mead water. To prevent the loss of the veligers, the water being removed was filtered in the cone portion of the plankton net with 64 µm pore size and the veligers were placed back into the corresponding tank. Each tank received at the minimum 25 veligers per liter of water (25 × 25 l = 625). After the veligers were added, the appropriate EarthTec® concentration was added to the treatment tanks. Because EarthTec® is considered a low pH product, the pH of the water in all tanks was recorded (APPENDIX 2).

Specimen Collection

As described in the toxicity tests, veligers were collected from Lake Mead, NV and brought to NDOW's hatchery in Boulder City, NV. The water temperature of Lake Mead was recorded each sampling day (APPENDIX 3).

The concentration of veligers was assessed by taking one ml of veliger sample and pipetted into a Sedgewick Rafter Grid (PhycoTech, Inc) and the veligers present in the grid were multiplied by the amount (ml) of the original sample. Three one ml samples were taken from each container and the average was calculated. For example, there are 20 veligers per one ml of 600 ml sample; therefore, 12,000 veligers are present in that sample (APPENDIX 4).

Phase I and Phase II Dosages

For both phases of the colonization project, a 10,000 ppm dilution of EarthTec® was prepared. The dilution was kept in clean glassware, covered, and away from moisture.

The following tables (4 and 5) outline the concentrations that correlate with the target ppm that were used in Phase I and Phase II of experimentation. After the first week in both phases, the EarthTec® dilution was divided in half to accommodate the water exchange.

Table 4 Phase I colonization experimental design

Date	EarthTec® Dosage for 1 ppm (ml)
12/1/2010	2.5
12/9/2010	1.25
12/15/2010	1.25
12/23/2010	1.25
12/29/2010	1.25

Table 5 Phase II colonization experimental design

Date	EarthTec® Dosage for 2 ppm (ml)	EarthTec® Dosage for 3 ppm (ml)
1/6/2011	5	7.5
1/12/2011	2.5	3.75
1/19/2011	2.5	3.75
1/24/2011	2.5	3.75
2/2/2011	2.5	3.75

Substrate Analysis

After four weeks, a substrate was removed from a control tank to assess colonization. The Olympus microscope with CPL microscopy was used. When colonization on the control substrate was present, one substrate from each of the 12 tanks was removed. To count the amount of colonization, all six surfaces of the substrate was observed. Once all the veligers that colonized were counted, the total number of mussels was divided by

0.0103 to determine the number of mussels per m^2. Each veliger was recorded and the substrate was brought back the UNLV's Environmental and Occupational Health Laboratory and photographed with the Zeiss Discovery.V8 stereo microscope (Carl Zeiss,Inc., Peabody, MA).

The colonization project was not held in a temperature controlled room. Therefore, accounting for evaporation was pertinent because the EarthTec® chemical may concentrate and increase in potency. Every two weeks, the loss of water was measured and the appropriate amount of EarthTec® was added back into each tank (APPENDIX 5).

Statistical Analysis

All statistical analyses above were performed using SAS® Software (version 9.2, SAS Institute Inc. Cary, NC).

CHAPTER 4

FINDINGS OF THE STUDY

Analysis of Data

Adult and Juvenile Toxicity

Toxicity tests of EarthTec® were performed with different stages of quagga mussels: adults (shell length > 11 mm; mean 17.99 mm ± 3.51, N = 240), juveniles (shell length < 11 mm; mean 7.48 mm ± 1.87, N = 240), and veligers (mean size 178.30 µm ± 56.59, N = 23).

Analysis of covariance (ANCOVA) showed that the concentrations of EarthTec® significantly affected the survival of adult mussels with time as a significant covariant ($p < 0.0001$). Higher concentrations of EarthTec® resulted in lower numbers of mussel survival and the increased time led to higher numbers of mussel mortality. No mortality occurred in the control groups for the adult mussels. The time to 100% mortality of adult *D. rostriformis bugensis* decreased with increasing EarthTec® concentration (Figure 4). Similar results were found among juvenile mussels (Figure 5): higher concentrations of EarthTec® and the increased time both led to a higher mortality rate (ANCOVA, $p < 0.0001$).

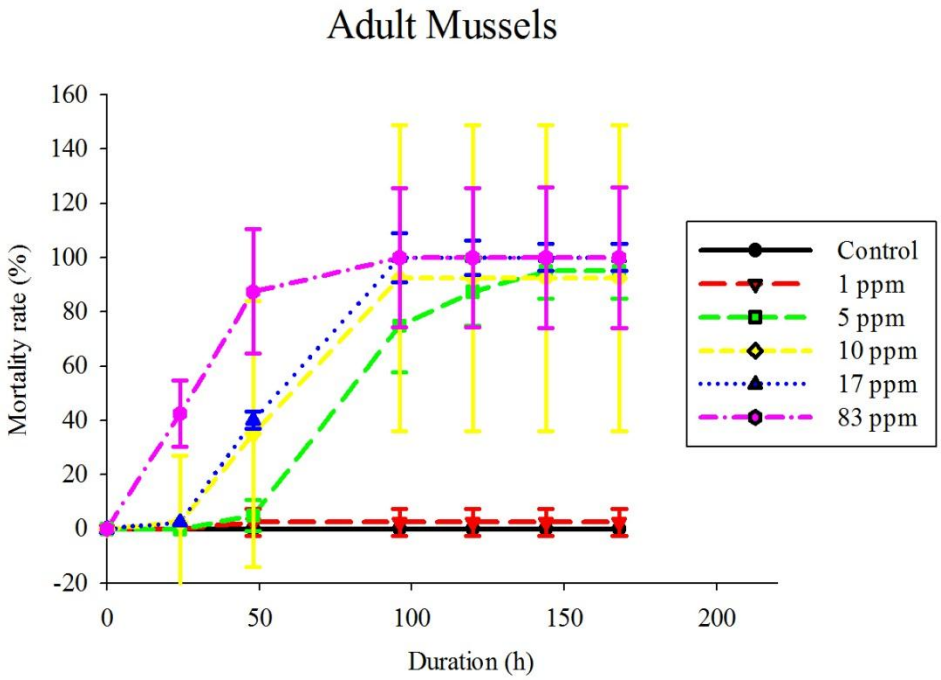

Figure 4 Cumulative mortality of adult *D. rostriformis bugensis* in six different EarthTec® concentrations (n = 240)

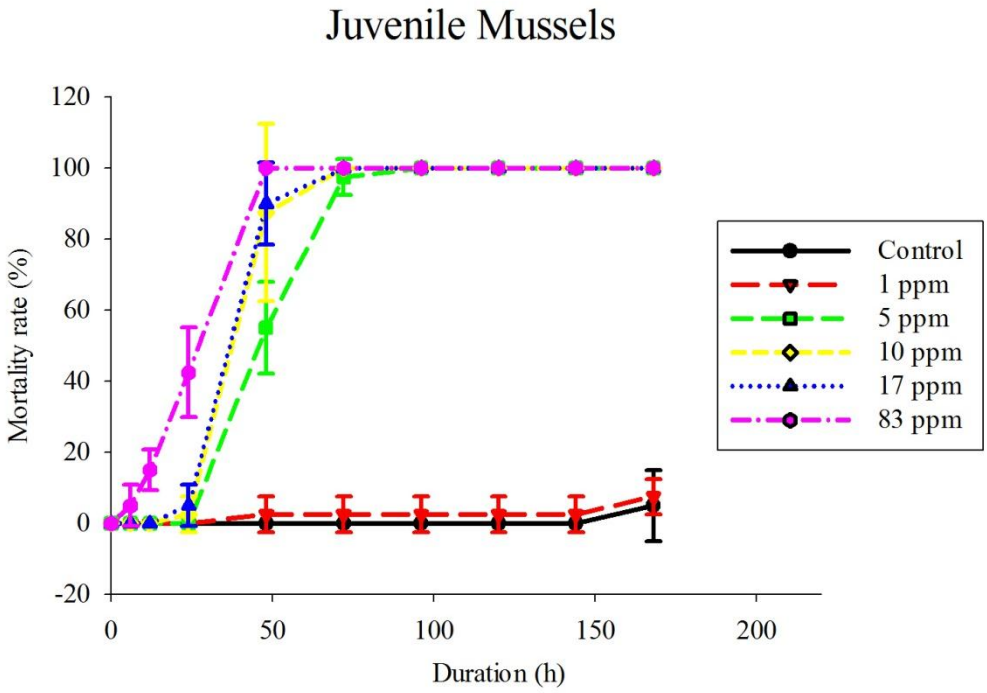

Figure 5 Cumulative mortality of juvenile *D. rostriformis bugensis* at different EarthTec® concentrations (n = 240)

In both the adult and juvenile toxicity tests, the control groups and the 1 ppm groups showed high survival rates. In the adult toxicity test, 50% of the mussels in the 83 ppm group of EarthTec® were dead by 30 h. By 96 h, >50% of the mussels in the group with 5 ppm were dead, >50% of the mussels in the group with 10 ppm were dead, and all the mussels in the 17 and 83 ppm groups were also dead. By 168 h, 5% of the mussels in the 5 ppm group were still alive and 7.5% in the 10 ppm group were still alive (Figure 4).

In the juvenile toxicity test, 5% of the mussels in the 83 ppm group were dead by 6 h. By 12 h, 5% more in the same group were counted as dead. By 24 h, less than half the mussels were remaining in the 83 ppm group, and 5% were dead in the 17 ppm group.

By 48 h, all the mussels in the 83 ppm group were dead, 90% were dead in the 17 ppm group, 87% were dead in the 10 ppm group, and almost half of the mussels were dead in the 5 ppm group. By 72 h, all the mussels in the 17 and 10 ppm groups were dead, 98% in the 5 ppm group were dead, and 3% were dead in the 1 ppm group. By 96 h, all the mussels were dead except for the controls and the 1 ppm group. No change in mortality was observed in hours 120 and 144. By 168 h, 5% of the control groups and 1 ppm groups were dead (Figure 5).

Veliger Toxicity

All doses, 3, 5, 10, 17, and 83 ppm were effective in killing 100% of the veliger *D. rostriformis bugensis* within minutes. Groups with 83, 17, and 10 ppm died in less than ten minutes. The groups with 5 ppm and 3 ppm died in less than 20 and 30 minutes, respectively, to die (Table 6). The experiment was completed after 36 h, and all the controls and individuals in the groups with 1 ppm were still alive. To reach a 100% mortality rate, the minutes needed were significantly different (ANOVA, $p < 0.001$). Student-Newman-Keuls multiple comparisons showed that the time to 100% mortality was significantly longer when treated with 3 ppm and 5 ppm than with higher doses such as 10 ppm, 17 ppm, and 83 ppm (Table 6).

Table 6 Time for veliger quagga mussels to reach 100% mortality at different doses of EarthTec® (n = 580)

EarthTec®	Minutes (Mean	±	Standard deviation)	Replicates
0ppm	*	±	*	8
1ppm	*	±	*	6
3ppm	27.5	±	7.5	4
5ppm	20.3	±	8.1	3
10ppm	6.0	±	2.0	3
17ppm	6.0	±	1.0	3
83ppm	5.7	±	4.5	3

* Mortality rate was 0

Colonization

For data obtained in Phase I of the colonization experiment, a pooled t-test was performed. The groups with 1 ppm of EarthTec® had less colonization compared to the control group ($p > 0.01$) (Figure 6).

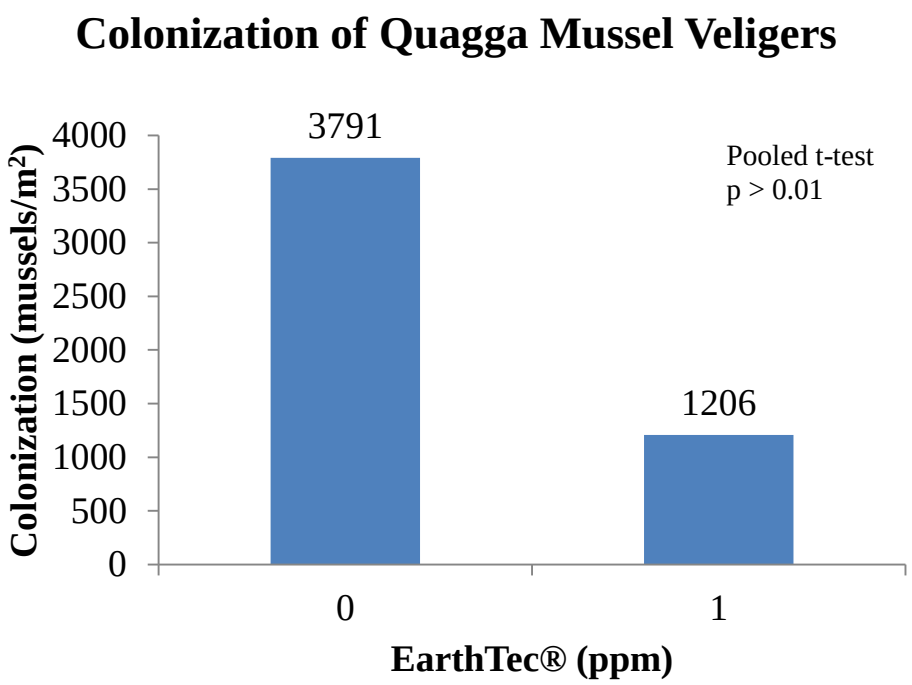

Figure 6 Density of quagga mussel colonization for 0 and 1 ppm of EarthTec® (N = 7994 mussels/m^2)

For Phase II of the colonization experiment, the treatments with 3 ppm of EarthTec® had a zero colonization rate (Figure 7). The groups treated with 2 ppm and 3 ppm were less colonized than the control group (ANOVA $p < 0.01$) (Figure 7), while there was no significant difference between 2 ppm and 3 ppm (Student-Newman- Keuls multiple comparison, $p > 0.05$).

Assuming the control (0 ppm) treatment had a 100% colonization rate in Phase I, an average 32% colonization rate was found for 1 ppm. The same assumption was applied for the Phase II experiment where the colonization rates for 0 ppm, 2 ppm, and 3 ppm treatments were 100%, 7%, and 0%, respectively. Therefore, EarthTec® with 1 ppm, 2 ppm, and 3 ppm had reduced colonization rates by 68%, 93%, and 100%, respectively. Because both control treatments had a 100% colonization rate, a linear regression was

used to determine the relationship between colonization rate (%) and dose (ppm) (Figure 8). It was found that the Colonization Rate = -32.5 × Dose + 83.4 ($R = 0.92$, $p < 0.05$). Therefore, to prevent colonization by quagga mussels, it is estimated that 2.6 ppm of EarthTec® is necessary.

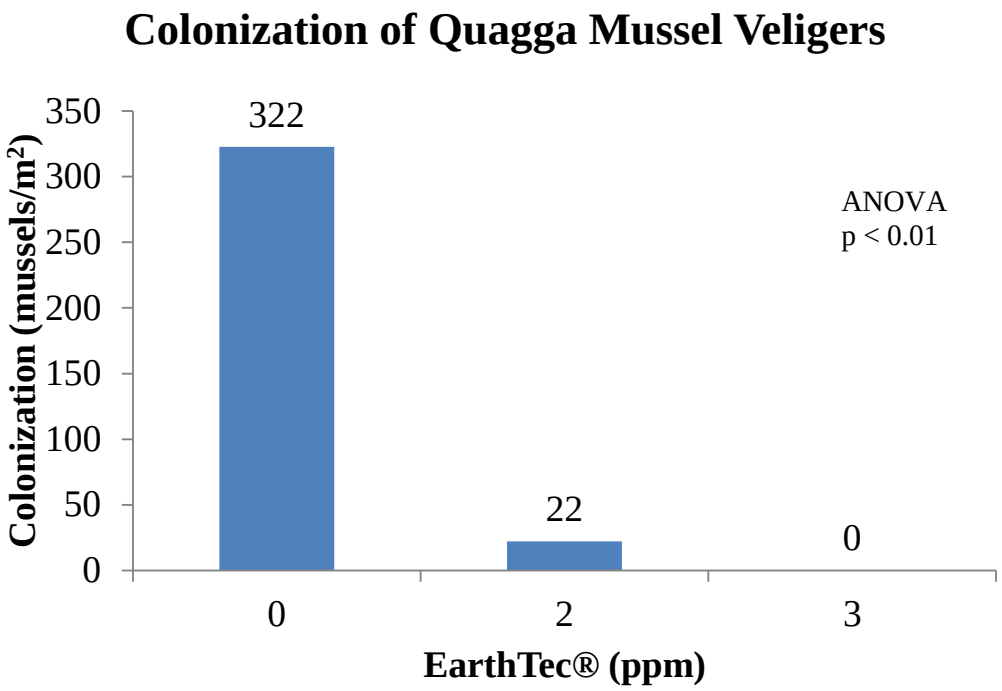

Figure 7 Density of quagga mussel colonization for 0, 2, and 3 ppm of EarthTec® (n = 344 mussels/m^2)

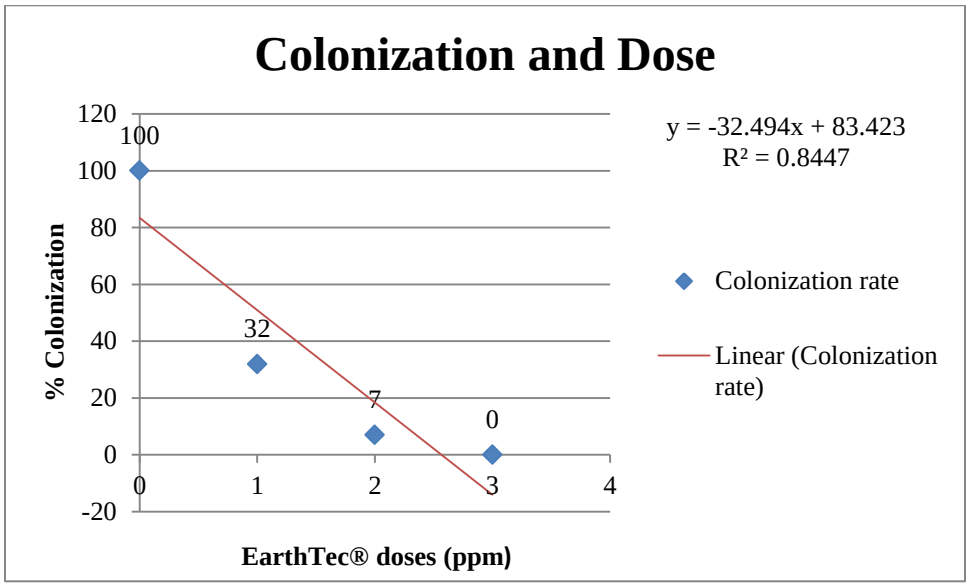

Figure 8 Relationship between the percent colonization rates and EarthTec® dose in quagga mussel colonization

CHAPTER 5

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

Discussion of Results

The first portion of the study examined the lethality of EarthTec® on quagga mussels. It was discovered that higher concentrations of EarthTec® and longer exposures were required for 100% mortality in adult mussels compared to juveniles or veligers. To kill over 50% of the mussels by 96 h, at least 5 ppm of EarthTec® (0.30 ppm Cu^{2+}) was needed. For 100% mortality of adult mussels, 5 ppm was administered over 168 h. For most water facilities that may choose to use EarthTec®, this may be too costly and time consuming. Depending on the location and the current amount of copper already in the water source, this amount of EarthTec® may be too high to use in a facility that treats drinking water. According to Earth Science Laboratories (2010), it is recommended to use 1 gal of EarthTec®/ 1,000,000 gal of water to control for algae and bacteria in a body of water. Field tests are needed to determine the amount needed to control for quagga mussels. Unlike chlorine, EarthTec® does not need to be applied continuously, so the dose and time may be reduced.

The results of this study suggest that EarthTec® is more effective in killing adult and juvenile quagga mussels than another algicide/bactericide/cyanobactericide, Cutrine®-Ultra. Cutrine®-Ultra is a chelated copper formulation that is effective in penetrating thick cell walled algae and vascular aquatic plants (Applied Biochemists, 2002). When adult zebra mussels (*D. polymorpha*) were exposed to 1,214 µg Cu/L (1.2 ppm Cu) for 48 h, only 50% mortality was achieved (Kennedy et al., 2006). This amount of copper is slightly below the US EPA's MCL of 1.3 ppm. After 96 h of continuous exposure, it

took almost two times the maximum allowable dosage of Cutrine®-Ultra to kill most of the adult zebra mussels. Another study also examined the toxic effects of copper sulfate ($CuSO_4$) on adult zebra mussels. Researchers used adult zebra mussels (shell length 20 – 25 mm) and found them to be resistant to copper, resulting in a 48 h LC_{50} of 5.3 ppm Cu/L, but the LC_{50} fell to 2.5 ppm Cu/L after continued assessment of survival after mussels were transferred to untreated water (Waller et al., 1993).

In the juvenile toxicity portion of the present study, most of the mussels were dead by 48 h using 5 ppm of EarthTec®. These results are similar to what was found in the adult toxicity portion, however, time was cut in half. It took 72 h for 100% mortality for juvenile mussels exposed to 5 ppm of EarthTec® (Cu^{2+} 0.3 ppm). This would cut the cost and time in half for a facility that may use EarthTec® to control juvenile sized mussels. Waller et al. (1993) found the LC_{50} for juvenile zebra mussels (shell length 5 – 8 mm) after 48 h of continuous exposure greater than 2 ppm of $CuSO_4$. This amount exceeds the MCL set by the US EPA at 1.3 ppm of copper, and only 50% of the sample was affected by the lethal concentration.

EarthTec® was found to be very toxic to veliger quagga mussels. Veligers do not have a protective shell; therefore, the Cu^{2+} can easily disrupt membrane function. Through this study, 3 ppm of EarthTec® (0.18 ppm Cu^{2+}) was found to be effective in killing veliger quagga mussels in minutes. Based on personal observation, EarthTec® was effective on all life stages of veligers, from trochophores to pediveligers. While the sizes of the veligers were recorded, the rank of veliger size at time of death was not recorded (Table 7). Kennedy et al. (2006) found the highest 24 h LC_{50} value for the early life stages (trochophores) of veligers to Cutrine®-Ultra was 13 µg Cu/L (0.013 ppm Cu).

The study showed that this chemical is effective in killing trochophores; however, it did not investigate the larger life stages of the veliger. Veligers are considered trochophores for up to three days. For Cutrine®-Ultra to be most effective, the exact time of spawning needs to be understood in order to use the least amount of chemical which will reduce the environmental effects and cost. One study examined the effects of copper ($CuSO_4$) on released mussel glochidia of several unionoid species. They found a range of copper toxicity at 24 h LC_{50} to be between 0.03 – 0.08 ppm Cu (Jacobson, Neves, Cherry, & Farris, 1997). Glochidia may be more sensitive to copper toxicity compared to quagga mussels.

Table 7 Sizes and developmental stages of veligers in toxicity experiment (n = 23) (mean size 178.30 µm ± 56.59)

Size (µm)	Life stage	Size (µm)	Life stage
154.32	Umbonal	92.62	Trochophore
238.96	Pediveliger	208.47	Umbonal
201.81	Umbonal	194.3	Umbonal
232.57	Pediveliger	238.01	Pediveliger
180.01	Umbonal	143.71	Umbonal
89.34	Trochophore	160.09	Umbonal
81.92	Trochophore	211.97	Pediveliger
186.32	Umbonal	148.7	Umbonal
243.08	Pediveliger	225.37	Pediveliger
177.77	Umbonal	256.08	Pediveliger
103.78	Trochophore	88.95	Trochophore
240.87	Pediveliger		

The second portion of the study examined the effects of EarthTec® on preventing quagga mussel veligers from colonizing fiberglass substrates. In both Phase I and Phase II, EarthTec® was effective in preventing veliger colonization. Phase I of the

colonization experiment showed that a greater density of mussel colonization occurred in the control groups whereas there was far less colonization in the 1 ppm group ($p > 0.01$). Mussel colonization was successfully deterred when veligers were exposed to 1 ppm of EarthTec® (0.06 Cu^{2+}). Phase II of the colonization study was conducted in January, where water temperatures were lower (APPENDIX 3). It was found that there was very little colonization in the 2 ppm groups compared to the control groups and no colonization in 3 ppm groups.

Presently, chlorine is the most commonly used chemical for prevention of veliger mussel colonization. One study that was conducted in a field laboratory found that an intermittent 2 h daily treatment with 1 mg/L (1 ppm) chlorine reduced mussel settlement by 91% as compared with the controls. Although, chlorine is effective in preventing quagga mussel veligers from settling, densities of up to 6000 m^2 still occurred compared to the control settling monitors which reached 147,100 m^2 (Bidwell et al., 1999). The same study also looked at using half the amount of chlorine (0.5 mg/L) for 4 h/day, and similar reductions in mussel colonization were found. The 2 to 4 h chlorine treatments did cause a reduction in settling, but the breaks in treatment were sufficient for the veligers to feed and grow (Bidwell et al., 1999). This intermittent chlorine schedule in this study may work for a while; however, it will not prevent mussels from fouling.

The present study with EarthTec® was conducted in a laboratory setting, and it cannot be assumed that the same results would occur if conducted in the field. If a water treatment facility were to consider using EarthTec®, it is important to understand the health implications that arise with copper toxicity. The US EPA set the MCL of copper in drinking water at 1.3 ppm (US EPA, 1991). As of 2009, the average level of copper

detected in the Southern Nevada Water Authority (SNWA) distribution system was 0.8 ppm (SNWA, 2010). If the SNWA chooses to use 1 ppm of EarthTec® (0.06 ppm Cu^{2+}) or even 2 ppm of EarthTec® (0.12 ppm Cu^{2+}) to deter veliger settlement, the copper output would still be below the US EPA's MCL.

Copper is an essential micronutrient and is required by the body in very small amounts. However, excess copper in the body can cause stomach and intestinal distress such as nausea, vomiting, and stomach cramps (Pizarro, Olivares, Uauy, Contreras, Rebelo, & Gidi, 1999). The chronic effects of copper, which are rare, can result in liver damage (de Romaña, Olivares, Uauy, & Araya, 2011). High levels of copper exposure usually occur from food and water ingestion, however, the intake does not pose adverse health effects because the body effectively regulates copper absorption, storage, and excretion (de Romaña et al., 2011). People with Wilson's disease, a rare genetic disorder, are more sensitive to the effects of copper.

Discussion of Research Questions

This study was designed to examine five questions pertaining to the effectiveness of the US EPA-registered and NSF-certified algicide/bactericide, EarthTec® on killing quagga mussels and preventing colonization of quagga mussel veligers. The first question examines whether or not EarthTec®, can be used as an effective molluscicide. Based on the toxicity test results and the colonization results, EarthTec® is effective in killing quagga mussels and preventing colonization of veliger quagga mussels. To register EarthTec® as a molluscicide, Earth Science Laboratories would apply for registration through the US EPA. Scientists at the US EPA would determine if the

product label and intended uses of EarthTec® are effective. To be environmentally safe and economical, it is important to know what the lowest concentration of EarthTec® that is necessary to kill adult, juvenile, and veliger quagga mussels. To reach 100% mortality, adult quagga mussels would need to be continuously exposed to 5 ppm of EarthTec® (0.30 ppm Cu^{2+}) for 168 h. Juvenile quagga mussels will reach 100% mortality when exposed to 5 ppm of EarthTec® (0.30 ppm Cu^{2+}) after only 48 h of exposure. EarthTec® exerts toxic effects on veliger quagga mussels quite rapidly. It took less than 30 minutes for 100% mortality of veligers when exposed to 3 ppm of EarthTec® (Cu^{2+} 0.18 ppm). Determining the most sensitive life stage to EarthTec® was the next question that this study was designed to examine. Based on the vulnerability of the veliger's size and lack of a protective shell, the results showed that veligers are more susceptible to the toxic effects of EarthTec® compared to adult and juvenile quagga mussels. Finally, the second portion of the study was designed to determine if EarthTec® is effective in preventing veliger quagga mussels from colonizing, and if so, at what dose would be needed. Targeting veligers to reduce colonization in water treatment facilities is cost effective because the dose is low, and application time would be minimized. Based on this study, EarthTec® is effective in preventing veliger quagga mussels from colonizing. Statistical analysis showed that approximately 2.6 ppm can prevent veliger colonization under the experimental conditions. Gerstenberger et al., (2011) suggest that veligers are more fit in the months between November and January; hence a lower dose (< 2.6 ppm) should be able to prevent colonization in other seasons, where the water is warmer.

Study Limitations

This study clearly showed that EarthTec® is effective in killing quagga mussels and preventing veliger colonization. However, the results observed should not be assumed to apply in all situations because of the *in vitro* nature of the study.

The sizes of the veligers that were added into the tanks for the colonization portion of the study were never measured because the microscope at the hatchery did not have photo imaging software. In every weekly subsample, it was observed that the sizes of veligers ranged from trochophores to pediveligers. Once settlement was discovered at the beginning of Phase I, the plates were examined at the hatchery using the same microscope and measurements were not recorded. During the second Phase, the plates with settlement were brought back to the Environmental and Occupational Health Laboratory at UNLV, and data were collected on the sizes of the settled veligers.

Although veligers were present in January, their numbers were quite low. The water temperature of Lake Mead during Phase II of the colonization experiment may have been too low to have large enough sample sizes to add to the tanks weekly. Quagga mussels have been known to grow and thrive in low temperatures (6 °C – 9 °C), however, it is stressful (Wong & Moore, unpublished data). Removing veligers from the lake, and placing them into a 25 gallon tank, with low water temperatures, may have been stressful and this could explain the low densities of colonization, even among the controls, in Phase II of the experiment. It would be worthwhile to repeat this portion of the study in the spring or early fall when veliger abundance is high and water temperatures are suitable for healthy growth and settlement.

Through the course of the study, the actual copper concentration was never tested due to lack of equipment. The appropriate amount of EarthTec® was administered and it was assumed that the appropriate amount of Cu^{2+} distributed evenly. For the colonization experiment, the tanks were not in a temperature controlled room and evaporation occurred. It is unknown if EarthTec® volatilizes with the water evaporation, however, it is known that once it binds with an organism (i.e. the veliger), it becomes biologically inactive. When accounting for evaporation, small doses of EarthTec® were added back into the tanks (APPENDIX 5).

Study Contributions

There are numerous ways to prevent and control quagga mussel infestations. However, there has not been any one solution that has been proven 100% effective against eradication in environmentally safe doses. At the present time, chemical control may be the most cost effective way to battle quagga mussels. This study contributes to the understanding of chemical options that are available for quagga mussel control and prevention. To my knowledge, this was the first study to test the efficacy of EarthTec® as a molluscicide. The results show that EarthTec® is effective in killing adult, juvenile, and veliger quagga mussels and is effective against preventing veliger colonization. However, EarthTec® is not registered as a molluscicide at this time, but data obtained in this study could assist in the process.

Currently, the most commonly used chemical for mussel control is chlorine. While chlorine has been shown to be effective in preventing mussel colonization, it creates undesirable chlorinated by-products, such as THMs. The formation, speciation, and

concentration of chlorinated by-products in drinking water vary according to several factors related to water source characteristics, operational parameters during treatment, and drinking water distribution systems (Legay, Rodriguez, Sadiq, Sérodes, & Levallois, 2011). The MCL for THMs is 80 ppb (US EPA, 2010) and the average level of THMs found in the SNWA distribution system, as of 2009, was 68 ppb (SNWA, 2010). This is close to the drinking water standard threshold at 80 ppb (US EPA, 2010). SNWA operates two water treatment facilities, Alfred Merritt Smith Water Treatment Facility and River Mountains Water Treatment Facility. Approximately 260 million gallons of water a day flow through the Alfred Merritt Smith facility. The facility uses 0.1 mg/L of a gaseous form of chlorine to control for quagga mussels, and it costs approximately $700,000-750,000 a year. On the other hand, the River Mountains facility uses 0.7 mg/L of free chlorine to control for quagga mussels, and the facility generates the hypochlorite on site. It is estimated that 150 million gallons of water flows through the River Mountains facility a day, and their estimated budget is approximately $350,000-400,000 a year (Eric Wert, personal communication, April 18, 2011). Both treatment facilities use a continuous dose of chlorine and this is what led to the increasing amount of THMs. The THM amounts can decrease at SNWA's water treatment facilities by either replacing chlorine with EarthTec®, or using both chemicals intermittently.

Chlorine is heavily used for controlling and preventing colonization of quagga mussels. It is inexpensive, works in most raw water systems, is toxic in low concentrations, and quickly loses toxicity without bioaccumulating (Sprecher & Getsinger, 2000). However, chlorine has disadvantages as well. The transport and storage of chlorine can be hazardous, can be harmful to system components, and it

produces THMs (Sprecher & Getsinger, 2000). The most common THM found in drinking water is chloroform. People can be exposed to chloroform, or other THMs, through ingestion, inhalation, and dermal exposure through skin contact (Legay et al., 2011). The most common routes are through drinking water, warm showers, and swimming in chlorinated pools (Backer, Ashley, Bonin, Cardinali, Kieszak, & Wooten, 2000). THMs have been indirectly linked to a cancer in humans and animals (Cotruvo & Regelski, 1989).

EarthTec® is a safe alternative to chlorine and it can be another option of chemical control. It is effective in preventing veliger colonization in low doses, which fall well below the US EPA's MCL. Data collected from this study can be used towards control management plans in bodies of water that are already infested with quagga mussels or in bodies of water that are threatened with infestation.

Recommendations for Further Study

Chemical management strategies targeting early larval stages of quagga mussels are more likely to be cost efficient and less prone to non-target environmental impact than strategies aimed at controlling adults and juveniles. The toxicity experiment was conducted from late November to early February when the veligers are competent in colonization in Lake Mead (Gerstenberger et al., 2011). Therefore, a lower dose, such as 1 ppm, may still be effective in preventing colonization in other seasons when veligers are less competent. Since the veliger dynamics in Lake Mead vary by different seasons, more research in an annual duration is needed to determine the lowest dose of EarthTec® that can result in zero colonization. In that case, it can provide a cost-effective (minimum

dose of EarthTec® to prevent colonization of quagga mussels) and environment-friendly management tool for those agencies that are interested in dealing with invasive quagga mussels by using EarthTec®.

EarthTec® may be most effective in the summer time when water temperatures are higher. Copper toxicity increases with an increase in temperature and decreases at lower temperatures. Rao & Khan (2000) examined the toxicity of $CuCl_2$ on zebra mussels (*D. polymorpha*) with increasing water temperatures. The ambient water temperature was set at 15 °C, and it increased to 20 and 25 °C. A 48 h LC_{50} of 0.78 ppm $CuCl_2$ at 20 °C decreased to 0.24 ppm $CuCl_2$ at 25 °C. A similar effect occurred at 96 h exposure of LC_{50} of 0.5 ppm $CuCl_2$ at 20 °C reduced to 0.11 ppm $CuCl_2$ at 25 °C. If the surface water temperature of Lake Mead is expected to increase, the concentration of EarthTec® may need to be re-evaluated to reduce cost and the amount of chemical being used.

More research needs to be done in Lake Mead to have a better understanding of when quagga mussels spawn. When this research is available, the earliest larval form can probably be targeted for chemical control. This in turn would reduce the amount of chemical that is necessary for application; hence, reducing cost and the adverse impact on the environment. The significantly higher chemical sensitivity of veligers compared to adult and juvenile mussels has pertinent implications in the design and use of the chemical. Application of chemical controls in the environment is dependent on a couple of factors. First, the chemical needs to be effective against the target organism (i.e. quagga mussels) while not having an adverse effect on the non-target species in the environment. Chemical control plans need to be safe, practical, easy to implement, and cost effective.

The establishment of invasive species, especially quagga mussels, has had profound effects on the economy and the environment of the affected regions. Infested boat hulls are recognized as one of the major vectors for the transfer of marine non-indigenous species (Piola, Dafforn, & Johnston, 2009). For hundreds of years, copper has been used as an anti-foulant on ships' hulls. Antifouling biocides, such as copper, exert strong selective pressures on both the target pests and non-target organisms, favoring organisms with increased resistance (Piola & Johnston, 2008). This means that copper based anti foulants have the ability to select for the settlement and transport of invasive species with a high tolerance of metal pollution. It is unknown if quagga mussels will build a resistance to EarthTec®, which is why a monitoring program should be put in place if EarthTec® is to be used, to ensure its effectiveness. If a species develops a metal tolerance, it may further assist their establishment and spread to new habitats (Piola et al., 2009).

A more sensitive parameter of testing the effectiveness of a chemical is using the filtration rate as an endpoint rather than mortality (Kraak, Lavy, Peeters, & Davids, 1992). Decreasing the mussel's filtration rate can cause mortality through asphyxiation or starvation, however, the time to mortality may be increased, but the dose of the chemical may be decreased. It would be interesting for future research to test the effects of small doses of EarthTec® on filtration rates in quagga mussels.

There are other applications of EarthTec® than water treatment facilities. EarthTec® can safely be administered to any body of water that has a quagga mussel infestation. Utilities and industries, such as power plants and paper mills, which use water to cool their systems or generate power, may find EarthTec® to be beneficial if quagga or zebra

mussels are a problem. Future research is needed to find out how EarthTec® could be administered and at what dose. EarthTec® could also be used in smaller lakes, such as Lake Las Vegas and the Lakes in Desert Shores. Quagga mussels could easily be spread from Lake Mead to other bodies of water through boats or recreational equipment.

Conclusions

Invasive quagga mussels have caused economic problems for boat owners, the park services, and SNWA. Now that Lake Mead is infested, routine maintenance is necessary to avoid further damage. Quagga mussels are also detrimental to the surrounding ecosystem and are harmful to the native species by effectively removing zooplankton from the water column that limits food available to higher trophic levels of the food chain and by bioaccumulating contaminants. With their efficient filtering capabilities, quagga mussels remove suspended materials and nutrients from the water, making little or none available for native aquatic species that feed on the same nutrients (Claudi & Mackie, 1994). Because of quagga mussels' high fecundity, planktonic veliger stage, and ability to attach to substrates with byssal threads (Ram & McMahon, 1996), they have easily and swiftly spread to other lakes and reservoirs in the Lower Colorado River Basin. Several strategies have been employed to mitigate and control their spread. Using a copper based product, EarthTec®, this thesis examined its effectiveness on killing adult, juvenile, and veliger quagga mussels and also its effectiveness on preventing veliger colonization.

For the toxicity portion of the study, 5 ppm of EarthTec® (Cu^{2+} 0.3 ppm) was effective in killing 100% of quagga mussel adults by 168 h. It took 72 h for 100% mortality for juvenile mussels exposed to 5 ppm of EarthTec® (Cu^{2+} 0.3 ppm). For

veligers, 3 ppm of EarthTec® (Cu^{2+} 0.18 ppm) was effective in less than 30 minutes for 100% mortality. For the colonization portion of the study, 1 ppm of EarthTec® (Cu^{2+} 0.06 ppm) was effective in reducing veliger colonization on fiberglass substrates. To prevent veliger colonization, 2.6 ppm of EarthTec® is needed.

While chemical control of quagga mussels has been proven effective in laboratory studies and closed systems, the recommended higher doses required for adult and juvenile eradication restricts the use of harsh chemical-based strategies in field studies. The best way to combat this issue is to determine the most sensitive life stage and tailor management techniques to that specific life stage, and in this case, the veliger. This would optimize target efficacy while minimizing chemical release into the environment, risk to non-target species, and cost of EarthTec® that would be required.

APPENDIX 1

NATIONAL PARK SERVICE COLLECTION PERMIT

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

Name of principal investigator:

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

Name of institution represented:

University of Nevada - Las Vegas

Co-Investigators:

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

Project title:

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

Purpose of study:

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

Subject/Discipline:

Exotic / Invasive Animals

Locations authorized:

Quagga mussels will be collected from Las Vegas Boat Harbor

Transportation method to research site(s):

Samples will be collected with hand

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

n/a

Name of repository for specimens or sample materials if applicable:

n/a

Specific conditions or restrictions (also see attached conditions):

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

Recommended by park staff(name and title):

Reviewed by Collections Manager:

Yes ___ No ___

Approved by park official:

Date Approved:

10/20/2010

Title:

Chief, Resources Management

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

(Principal investigator's signature)

10/25/2010
(Date)

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

APPENDIX 2

pH VALUES FOR 12 TANKS IN COLONIZATION PROJECT

Tank	pH1	pH2	pH3	Mean
Control 1	8.28	8.25	8.25	8.26
Control 2	8.27	8.25	8.25	8.26
Control 3	8.27	8.26	8.26	8.26
Control 4	8.26	8.24	8.25	8.25

Tank	pH1	pH2	pH3	Mean
Treatment 2A	8.26	8.26	8.25	8.26
Treatment 2B	8.26	8.26	8.25	8.26
Treatment 2C	8.25	8.25	8.26	8.25
Treatment 2 D	8.26	8.25	8.25	8.25

Tank	pH1	pH2	pH3	Mean
Treatment 3A	8.24	8.24	8.24	8.24
Treatment 3B	8.24	8.24	8.24	8.24
Treatment 3C	8.24	8.24	8.24	8.24
Treatment 3D	8.24	8.25	8.24	8.24

APPENDIX 3

LAKE MEAD SURFACE WATER TEMPERATURES ON VELIGER COLLECTION DAYS

Date	C°	F°
December 1, 2010	13.8	56.8
December 9, 2010	14.1	57
December 15, 2010	14.6	58.3
December 23, 2010	12.3	54
December 29, 2010	13.8	56.8
January 6, 2011	9.6	49.3
January 13, 20100	13.3	55.8
January 19, 2011	13.2	55.7
January 24, 2011	12.5	54.5
February 2, 2011	9.7	49.4

APPENDIX 4

VELIGER COUNTS PER TANK IN COLONIZATION EXPERIMENTS

Date	Sample (ml)	1 ml pipette	Sample x 1 ml pipette	Average	Veliger Count	Veliger/Tank
12/01/2011	A. 600	6 7 6	3600 4200 3600	3800	300	~500
	B. 600	4 4 4	2400 2400 2400	2400	200	
12/09/2010	A. 600	20 20 20	12000 12000 12000	4000	333	~1370
	B. 300	25 15 15	7500 4500 4500	5500	458	
	C. 600	10 15 10	6000 9000 6000	7000	583	
12/15/2010	A. 600	6 9 6	3600 5400 3600	4200	350	~950
	B. 600	10 2 7	6000 1200 4200	3800	316	
	C. 600	6 9 2	3600 5400 1200	3400	280	
12/23/2010	A. 600	3 8 6	1800 4800 3600	3400	283	~950
	B. 600	6 3 10	3600 1800 6000	3800	316	

	C. 600	7 8 6	4200 4800 3600	4200	350	
12/29/2010	A. 600 B. 600 C. 600	4 8 7 7 4 9 8 6 7	2400 4800 4200 4200 2400 5400 4800 3600 4200	3800 4000 4200	316 333 350	~1000
1/06/2011	A. 600 B. 600 C. 300	4 6 2 4 6 5 10 10 10	2400 3600 1200 2400 3600 3000 3000 3000 3000	2400 3000 3000	200 250 250	~700
1/13/2011	A. 600 B. 600	7 7 6 7 10 8	4200 4200 3600 4200 6000 4800	4000 5000	333 416	~750
1/19/2011	A. 600 B. 600	12 14 17 9 4 20	7200 5600 10200 5400 2400 12000	7666 6600	638 550	~1670

	C. 600	15 3 11	9000 1800 6600	5800	483	
1/24/2011	A. 600 B. 600 C. 600	34 28 13 10 6 7 3 9 3	20400 16800 7800 6000 3600 4200 1800 5400 1800	10800 4600 3000	900 383 250	~1500
2/02/2011	A. 600 \ B. 600	1 1 1 1 1 0	600 600 600 600 600 0	600 400	50 30	~80

APPENDIX 5

WATER EVAPORATION

December 24, 2010

Tank	Water (L)	EarthTec® working solution (ml)
Control 1	2	0
Control 2	2	0
Control 3	2	0
Control 4	4	0
Control 5	2	0
Control 6	2	0
Treatment 1	2	0.2
Treatment 2	2	0.2
Treatment 3	2	0.2
Treatment 4	3	0.3
Treatment 5	2	0.2
Treatment 6	3	0.3

January 19, 2011

Tank	Water (L)	EarthTec® working solution (ml)
Control 1	3	0
Control 2	3	0
Control 3	2	0
Control 4	3	0
Treatment 2A	1	0.2
Treatment 2B	1	0.2
Treatment 3C	2	0.4
Treatment 2D	3	0.6
Treatment 3A	2	0.6
Treatment 3B	3	0.9
Treatment 3C	2	0.6
Treatment 3D	3	0.9

February 2, 2011

Tank	Water (L)	EarthTec® working solution (ml)
Control 1	5	0
Control 2	4	0
Control 3	3	0
Control 4	4	0
Treatment 2A	2	0.4
Treatment 2B	3	0.6
Treatment 3C	3	0.6
Treatment 2D	1	0.2
Treatment 3A	2	0.6
Treatment 3B	3	0.9
Treatment 3C	3	0.9
Treatment 3D	2	0.6

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