THE EFFECT OF COPPER ON THE MORTALITY AND REPRODUCTIVE VIABILITY OF ZEBRA MUSSELS

by

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THE EFFECT OF COPPER ON THE MORTALITY AND REPRODUCTIVE VIABILITY OF ZEBRA MUSSELS

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ABSTRACT

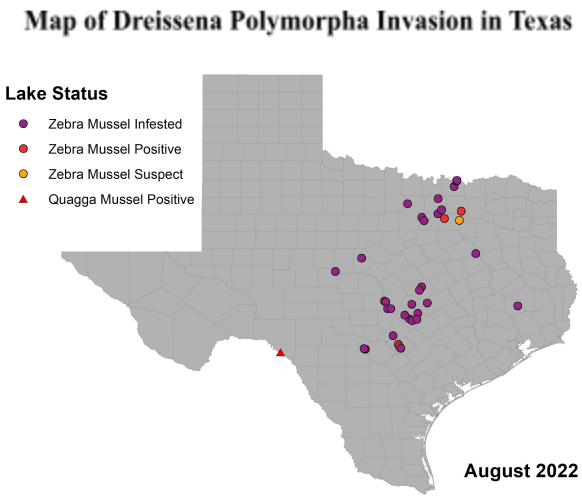
Zebra mussels are an invasive species that have infested many bodies of water, including Texas lakes and streams. They cause billions of dollars in infrastructure damage by clogging pipes and water intakes. They also have a significant ecological impact on aquatic ecosystems and native species. Zebra mussels can spread as adults by attaching to commercial boats or as planktonic larvae via water flow through lakes and rivers. Several methods are used to help prevent the spread of zebra mussels. Heated water, chlorine, and copper are currently used to help control the spread through pipes in factories and water treatment facilities. One promising copper solution, Earthtec QZ, was designed to treat algal blooms but was recently found to kill mollusks. The exact effectiveness of Earthtec QZ on eliminating zebra mussels is unclear. This project examines the effects of copper treatment on both adult zebra mussels and the developmental stages including gametes and fertilization. Adult mussels of varying size classes were exposed to different concentrations of Earthtec QZ to determine mortality rates. In another experiment, eggs and sperm were exposed to the copper treatments to determine its effects on fertilization. A third experiment tested chlorine concentrations previously found to kill veligers as an alternative.

INTRODUCTION

Dreissena polymorpha, also known as the zebra mussel, are freshwater bivalve shellfish originating from Russia and Ukraine. In 1988, they were introduced to United States waters and have since been labeled as an invasive species (Invasive Species Info). Invasive species are able to rapidly reproduce and overtake an ecosystem that is not their own, disrupting the other life forms (Herbert et al. 1989). Zebra mussels do this by using their byssal threads to attach themselves to hard surfaces such as boats, which allows for their quick spread, as well as pipes that run into cities, effectively preventing water flow through the buildup (National Park Service, 2021). They are aggressively invasive resulting in negative impacts ecologically. Zebra mussels compete with native mussel species in the area by attaching to them and draining them of their resources. Due to their filter feeding, they consume large amounts of the plankton floating in the water, taking away necessary nutrients from other species. This can cause displacement and alterations in the food web. They also attach to native mussels and suffocate them. Since they have few natural predators in the areas they invade, their growth continues unchecked.

A major factor contributing to their success as an invasive species is their reproductive biology. They reproduce rapidly by gamete release into the water. Sperm released by males can bind and fertilize eggs released by the females. The females can release up to a million eggs annually and start reproducing after two years (Ackerman et al, 1994). After fertilization, the larvae forms a shell composed of calcium and becomes known as a veliger. Byssal threads form two to three weeks later allowing surface attachment (Department of Natural Resources, 2023). This process allows the spread of zebra mussels in all stages of development, therefore giving them at least two weeks as a veliger to travel to various parts of the water body (Griffins et al 1991).

Zebra mussels can also have large negative economic impacts. They cause infrastructure damage by sticking to pipes and water intakes and clogging them. Their attachment to boats can also cause damage and sometimes destroy the engines. Due to their potentially harmful nature, zebra mussels can not be present in drinking water so laws have been passed to prohibit boats from approaching fresh water unless proper drainage and cleaning has been conducted (Texas Parks and Wildlife Department, 2022). In Texas, it is against the law to possess or transport zebra mussels, both dead and alive, due to their invasive nature. Unfortunately, it is not economically feasible to completely close infested bodies of water in order to try and treat them. In a 2007 study, the economic impact from zebra mussel invasion in North America on drinking water treatment and electric power generation facilities between 1989 and 2004 was $267 million (Connelly et. al, 2007). This does not include the cost of repairing other infrastructure or boats. Taking into account that mussel removal has been nearly impossible and they have continued to spread to countless other bodies of water, the economic impact of zebra mussel infestation today is much higher. Zebra mussels were first found in Texas in 2009 and, as of August 2022, infest six river bases and 30 lakes. They have been detected in three additional lakes however they have not yet invaded those ecosystems (Texas Parks and Wildlife Department, 2022). Tarrant County's own Lake Worth is currently infested and officials are looking for ways to decrease their numbers. The more populous they are, the harder it is to get rid of zebra mussels.



Texas Parks and Wildlife Department, 2022

Removing zebra mussels from a water source after their invasion is extremely difficult since they reproduce quickly and their veligers can travel far. In addition, it is unsafe to put certain chemicals in these ecosystems due to the harmful impact they can have on other species. Currently, a 2% bleach solution is effective for killing zebra mussels outside of water bodies and removing them from pipes and boats (University of Wisconsin Stevens Point). However bleach is one of the chemicals prohibited from water bodies at high concentrations, due its harmful effect on the other organisms.

This paper investigates using copper as a possible way to eradicate zebra mussel populations while not harming the ecosystems or humans in the process. EarthTecQZ is a molluscicide developed from the cupric form of copper, Cu^{2+}. This is the only active form of copper and due to the formulation of the solution, copper stays in its cupric form, allowing a smaller concentration to remain effective. Because the zebra mussels do not recognize the solution as a foreign invader, they readily absorb it. The Minnesota Aquatic Invasive Species Research Center found that using 1 ppm of copper has shown promise of controlling zebra mussel populations in their early stage trials, but research is still underway (University of Minnesota, 2023). The copper solution EarthtecQZ will soon be in use in Colorado by their Parks and Wildlife department in an attempt to control their mollusk population (Colorado Parks and Wildlife, 2023). Studies have shown that EarthTecQZ was effective in killing both zebra and quagga mussels (Watters et al 2013, Claudi et al. 2013). Finding the lowest concentration of Earthtec QZ that effectively destroys adult mussels would be of value to address treatment of any body of water that has become infested with *Dreissena polymorpha*. In addition, understanding how Earthtec QZ impacts the reproduction of zebra mussels can provide insight into the effectiveness of using it as a prevention method.

METHODOLOGY

Zebra mussels were collected from Lake Travis to use for the adult mortality and fertilization experiments. They were stored at 12°C in tanks and fed intermittently with Shellfish 1800 from Reed Mariculture. An artificial pond water was used for all mortality and spawning trials: 0.1 mM KCl, 0.7 mM $MgSO_4$, 0.8 mM $NaHCO_3$, 0.6 mM $CaCl_2$ (Dietz et al. 1994). This mimics the water present in lakes where zebra mussels are typically found and served as a negative control for untreated mussels.

EarthTec QZ served as the way to add copper into the pond water. These experiments test out different dilutions of EarthTec QZ to find the lowest dose to effectively impact zebra mussels. It was diluted into 4 different concentrations with the artificial pond water, as shown in the table below.

EarthTecQZ Concentrations			
Concentration	EarthTecQZ (uL)	Pond Water (mL)	Copper concentration
10 (0.17ppm)	10	600	0.01ppm
20 (0.34 ppm)	20	600	0.02ppm
30 (0.51ppm)	30	600	0.03ppm
60 (1.02 ppm)	60	600	0.06ppm

To test the breadth of the effect of Earthtec QZ, the mortality rates were observed in adults as well as the ability to spawn.

Adult Mortality Copper Trials

Before the trials began, adult zebra mussels were separated into size classes: small (S) 0.5 to 10 mm, medium (M) 15 to 20 mm, and large (L) >25 mm (Figure 1). Fifteen ounce (oz)

plastic containers were used to hold 10 mussels of the same size class. They were filled with cold water to acclimate to room temperature over 1-2 days. The pond water was then replaced with a solution of either a 0, 10, 20, 30, or 60 µL/600 mL concentration of Earthtec QZ. Containers were randomly dispersed. Tops with an airstone passing through allowed for a closed container with oxygen still flowing through. A hole was drilled on top for food distribution. Shellfish Diet 1800 was added to each container once a week and complete solution changes were done once per week. Mussels were checked every two days to record mortality. Mortality was based on gapping behavior of the mussels. When mussels die, they open their shells and are unable to close them due to non functioning ligaments and lack of muscular contraction. Thus, a mussel was determined dead if it was gaping open and did not close from tactile stimulus. Those that died were removed from the solutions to eliminate further contamination and placed in a bleach solution to properly dispose of the invasive species. These trials were repeated two additional times.

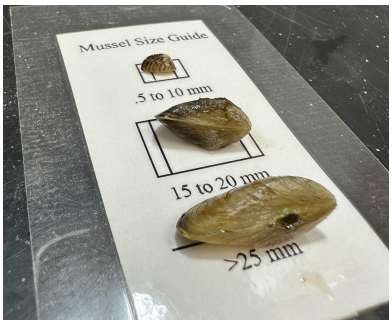


Figure 1. Three size classes of zebra mussels

Adult Mortality Chlorine Trials

Room temperature acclimation was repeated for 150 adult mussels: 50 small, 50 medium, and 50 large. They were placed into chlorine solutions with concentrations of 0 mg/mL, 0.5 mg/mL, 0.75 mg/mL, 1 mg/mL, and 2 mg/mL. To make these solutions, 50 g/L sodium hypochlorite was diluted 1:10 with DI water. Chlorine amounts of 60 µL, 90 µL, 120 µL, and 240 µL were added to 600 mL pond water to make the set concentrations respectively. Solutions were changed every 4 days and they were fed every 3 days. Mortality was observed daily.

Fertilization and Early Development Trials

Adults were taken out of cold tanks and acclimated to room temperature for 1-2 days. Spawning followed procedures of Misamore et al. (1996). Each male and female were separated into their own 25-ml, flat-bottomed glass tube and submerged in a solution of 1 mM serotonin (5-hydroxytryptamine) in pond water for 15 minutes. Males remained in the tubes once release of sperm (spawning) commenced. When females began releasing eggs, they were transferred to a 70 × 50 mm crystallizing dish containing approximately 50 mL of PW. Sperm from one male was isolated and 1 mL was put into all 5 glass tubes, each containing an EarthtecQZ concentration. After an incubation time of five minutes, 1 mL of eggs from the same female was added into each tube of sperm. Samples were taken at time points 5, 20, and 90 minutes and put into 1 mL 3.2% paraformaldehyde in mussel buffer (5.5 mM TAPS, 0.145 mM KCl, 0.8 mM NaCl, 0.8 mM Na_2SO_4, 0.89 mM $MgSO_4$, 1.32 mM $NaHCO_3$, 1.19 mM $CaCl_2$) (Misamore and Lynn, 2000). Fixation stopped the developmental process at specific timepoints and allowed for later observation. The samples remained refrigerated until used. Four independent trials consisting of different males and females were performed. The samples for each concentration at each time point were viewed using either a Zeiss Axiovert 200 equipped with epifluorescence optics or a Nikon Optiphot microscope phase contrast optics microscope. Digital micrographs were captured using a Zeiss AxioCam MRm and Axiovision software.

The number of bound sperm was determined at the five minute time point. The samples were placed on a microscope slide with vaseline at the corners and observed using a phase contrast microscope. To determine bound sperm, eggs were viewed at an equatorial focus, and sperm bound perpendicular to the egg surface were counted. The number of equatorial bound sperm was recorded for 30 eggs for each concentration.

To determine if sperm were able to fuse and enter into the egg cytoplasm, otherwise known as fertilization, the 20 min samples were examined for sperm incorporation. At 20 min, the egg should be fertilized and a polar body may start appearing. In order to see whether or not sperm have entered an egg, fluorescent microscopy was required. Samples were stained with the DNA-specific dye Hoeschst 33342. Each sample was washed with a mussel buffer and stained. To make the dye, 10 µL of 1mg/ml stock Hoescht 33342 solution was diluted with 1 mL DI water and mixed. Half of the sample was transferred into a new tube. Mussel buffer was added until it reached the 1 mL mark. Tubes were inverted twice and then left to sit to allow eggs to settle to the bottom. After 10 minutes, 0.5 mL was drawn off the top and discarded. This process was repeated to wash the sample again. After two washes, lights were turned off to begin sample staining and 0.5 mL was drawn off from each sample. They were each refilled to 0.9 mL with the mussel buffer. To reach a final concentration of 1 µg/mL, 100 µL of diluted stain was added to each sample. The samples were placed in a dark box and sat for 15 min. Following staining, the samples were washed twice by half volume changes using the mussel buffer. After the second wash, the samples were drawn down to 0.5 mL and kept in the dark. Samples were put on a microscope slide with vaseline and viewed under the fluorescent microscope.

To determine if fertilized eggs continued the developmental process by dividing, the 90 minute time point was examined. Fixed samples were placed on a microscope slide with vaseline at the corners and observed using a phase contrast microscope. To determine whether or not cleavage had occurred, eggs were viewed at an equatorial focus and the number of eggs that had divided were counted. Cleavage was recorded for 30 eggs for each concentration.

RESULTS

Adult Mortality

When looking at mortality of adult mussels, EarthTecQZ concentrations were examined over a six day period. The number of mussels in all three trials that survived on the sixth day were graphed and a regression line was determined. Each dot on Figure 2a. represents one mussel, however most of the data overlapped. Using the regression values, P was found to be 0.0746. Since $p > 0.05$, the effect of copper solutions on mussel mortality was not found to be significant. However, there was one trial in which the mussels in the 60 µL/600 mL did not die like they did previously. The rest of the concentrations in this trial still produced similar results and all died by day six. When graphing the data without the use of the 60 µL/600 mL in Figure 2b, $P = 0.009$. Since $p < 0.05$, the results were significant and EarthTec concentrations tested did have an effect on the mortality of zebra mussels at day six.

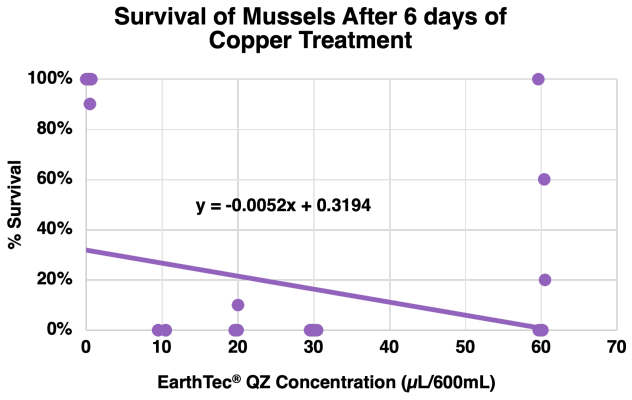


Figure 2a. Mortality of 450 mussels after 6 days at different EarthTecQZ concentrations

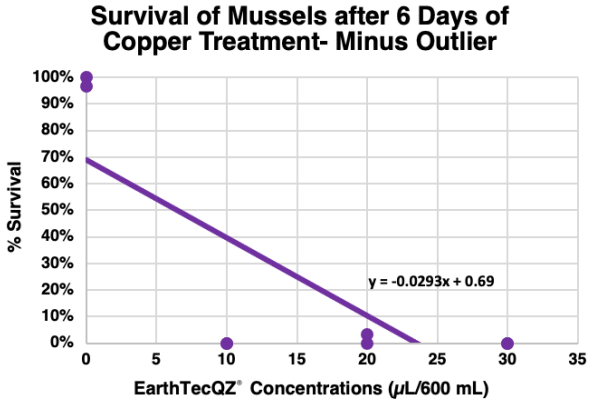


Figure 2b. Mortality of 450 mussels over 6 days at different EarthTec QZ concentrations without the 60 outlier

In addition to day six mortality, mussels were analyzed over the six day period to determine if the EarthTecQZ affected the survival rate over time. A Mantel Cox Test was performed to analyze the probability of survival for different copper concentrations. Figure 3 shows three trials of mussels from all concentrations graphed with standard deviation. Mussel

survival was recorded over the 6-day time period. There was a statistically significant difference in survival probability between the treatments ($p<0.001$).

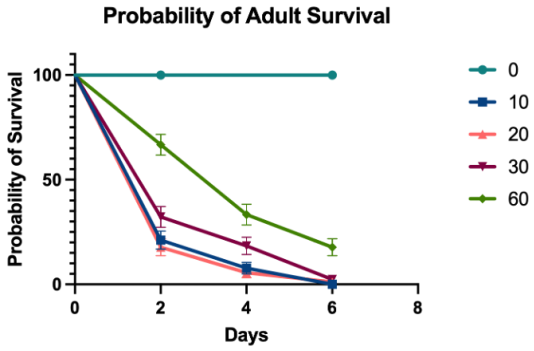


Figure 3. Mantel Cox Test, survival of all three trials graphed in two days intervals. Bar-standard deviation

Size was also considered when examining the effects of EarthTecQZ. At each concentration, the probability of survival for small, medium, and large were graphed with standard deviation in Figure 4. At 10 µL/600 mL, there was a significant difference in survival probability between the size classes ($p<0.0089$). In pairwise comparisons, there was a significant difference in survival probability between the small and medium sized mussels ($p< 0.05$). There was no significant difference in survival probability between the medium vs large or the small vs large mussels ($p>0.05$). At 20 µL/600 mL, size did impact survival probability ($p = 0.002$). There were significant differences between all three size classes ($p<0.05$). At both 30 and 60 µL, there was no significant difference in survival probability between the size classes ($p = 0.2528$ and $p = 0.3001$ respectively). This indicates there was no impact of size on mortality over time in either concentration.

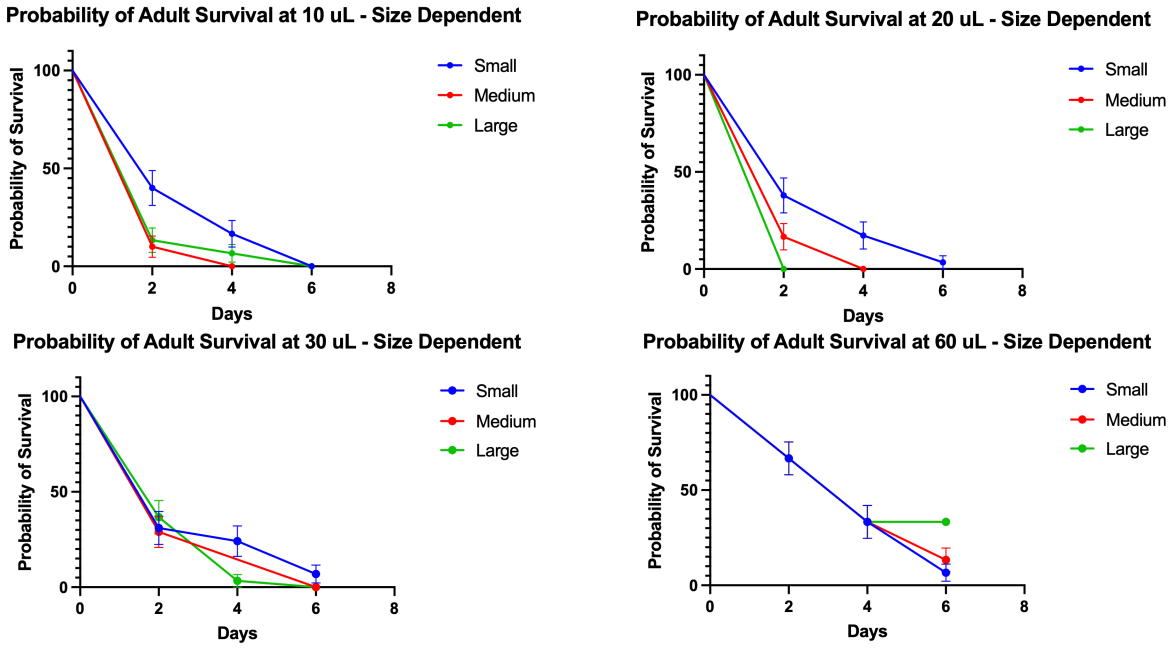


Figure 4. Probability of survival over time for each size class in all four EarthTecQZ volumes tested

Adult Mortality Trials with Chlorine

The effects of low-dosage chlorine on adult mussel survival was tested. Survival was greater than 90% in all chlorine treatments. There was no significant difference in probability of survival based on a Mantel Cox Test ($p = 0.9256$) (Figure 5). These results show that there was no significant difference in mussel survival from the use of chlorine solutions on adult zebra mussels.

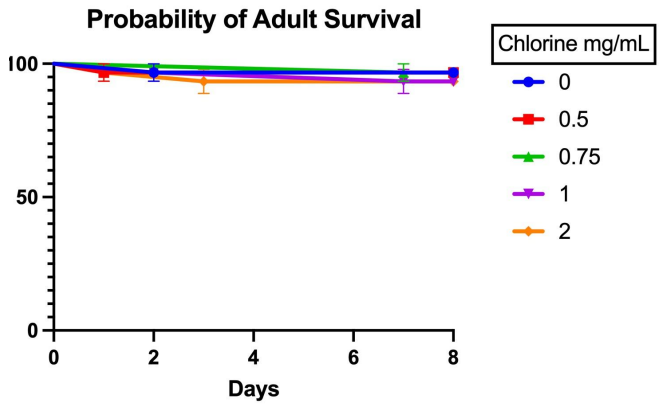


Figure 5. Mantel Cox probability of survival plot for the varying chlorine concentrations. There was no significant difference in survival between treatments (p=0.9256)

Fertilization Trials - Sperm Binding at 5 Minutes Post Insemination

At five minutes post insemination, sperm are expected to bind to the egg. Figure 6a. demonstrates what an egg with two sperms bound looks like under a phase contrast microscope as well as an egg with no bound sperm. This form of identification was used to record the number of eggs with bound sperm. Results from 30 eggs per concentration were averaged and graphed. Figure 6b. shows the average eggs with bound sperm for each concentration with standard deviation. There was no significant difference in sperm binding between the control and EarthTec QZ concentrations based on a one way analysis of variance (ANOVA), $F(4,19) = 1.289$ ($p = 0.3184$). Since $p > 0.05$, the results are not significant and there is no difference in the number of eggs with bound sperms between the negative control and EarthTec QZ concentrations.

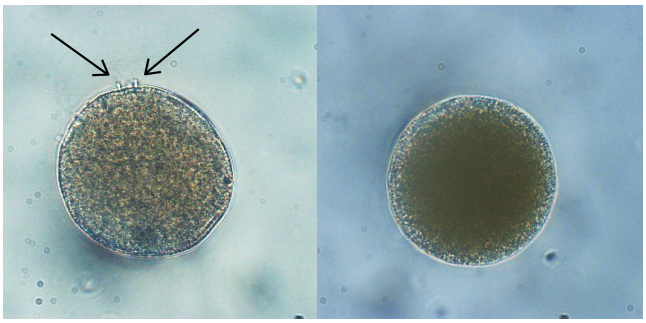


Figure 6a. Egg fixed at 5 min show presence of bound sperm (Concentration 10). Left: Egg with 2 bound sperm (arrows), Right: Egg with no bound sperm.

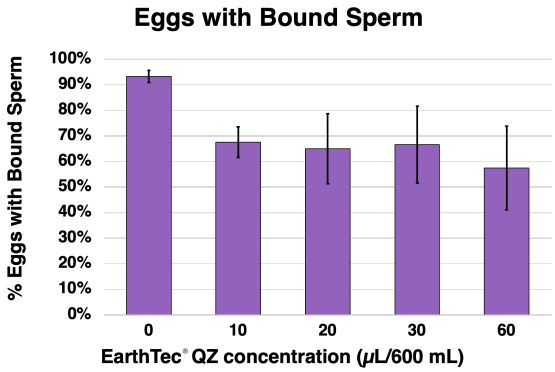


Figure 6b. Average numbers of bound eggs vs EarthTec QZ concentration. Bar- SE

In addition to these quantitative results, qualitative observations were made. Eggs appeared to lose their circular shape in the axis view and some burst open as the EarthTecQZ concentration increased. This is shown in Figure 7, captured at 20 µL/ 600 mL. Sperm accumulated around the egg and intermixed with the released egg contents. Burst eggs were observed in the various EarthTecQZ concentrations but not in the control treatment.

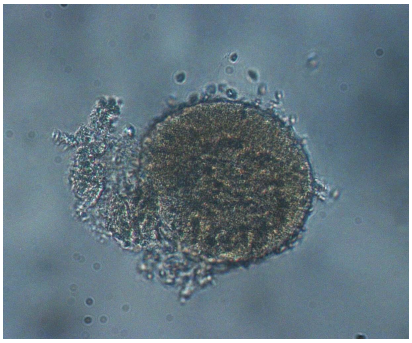


Figure 7. Example of egg that burst when exposed to EarthTec QZ.

Fertilization Trials - Sperm Entry at 20 Minutes Post Insemination

Twenty minutes after insemination, sperm should be inside of the egg, indicating fertilization. Polyspermy is common in zebra mussels so it is not uncommon for multiple sperm to get inside an egg (Misamore et al., 1996). A polar body may or may not be present as well, depending on the development stage. Figure 8a. shows an egg with a sperm inside and the presence of its own genetic material. This time point aimed to see if EarthTecQZ affected fertilization. The number of fertilized eggs out of 30 from four trials were averaged and graphed

with standard deviation in Figure 8b. There was a significant difference in sperm entry between the treatments based on a one way analysis of variance (ANOVA), $F(4,19) = 3.757$ ($p = 0.0281$). Based on multiple comparisons, only the 60 µL/600 mL concentration had a significant impact on the percent of fertilized eggs relative to the control (Figure 8b).

Figure 8a. Corresponding fluorescent (left) and phase contrast (right) images of a fertilized egg. Sperm nucleus is visiable in the egg cytoplasm (arrow) as well as the female DNA dividing during completion of meiosis (arrow).

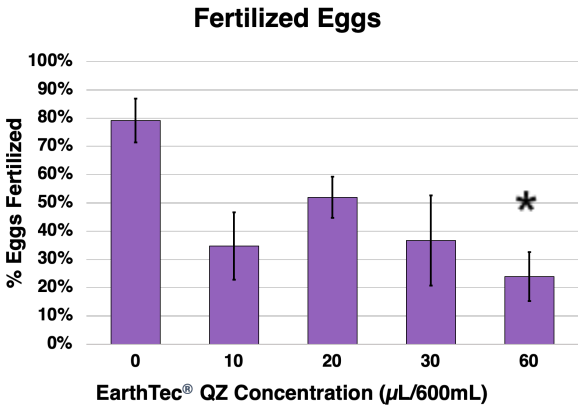


*Figure 8b. Mean percentage of fertilized eggs over four trials is shown for each EarthTecQZ concentration. Bar-SE. *-indicated significant difference.*

Observations were also made that suggested a similar phenomenon shown in the 5 minute trials. Some eggs at each EarthtecQZ concentration looked like there was an excess of sperm around and potential spillage from the membrane bursting open. The DAPI dye identified part of the material around the egg as sperm in Figure 9. This data was not quantified but was identified in concentrations 10, 20, 30, and 60 µL per 600 mL.

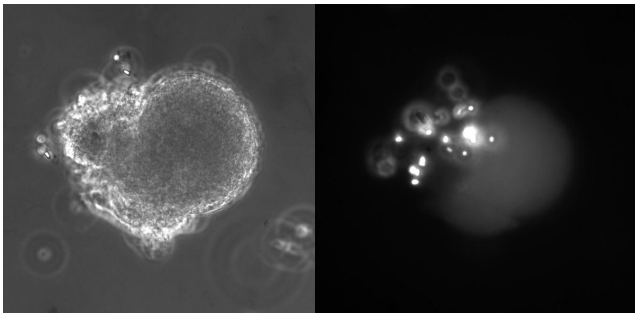


Figure 9. Corresponding phase contrast (right) and fluorescent (left) images of a burst egg at 20 min postinsemination.

Fertilization Trials - Cleavage at 90 Minutes Post Insemination

Ninety minutes after spawning occurs, the egg should be in the process of cleaving. Figure 10a. shows a cleaved egg and an uncleaved egg. Thirty eggs were observed for each concentration with cleavage recorded and mean numbers of dividing eggs determined. There was a significant difference between the mean percentage of eggs undergoing cleavage between the treatments using a one way analysis of variance (ANOVA), $F(4,19) = 12.73$ ($p < 0.0001$). Multiple comparisons show a significant difference in cleavage between the control and all EarthTec treatments, but no significant difference between each EarthTec treatment ($p < 0.05$).

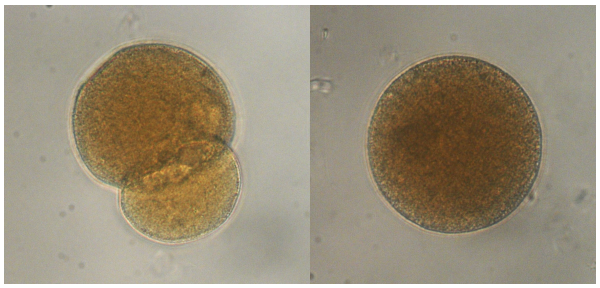


Figure 10a. Phase contrast micrograph showing a cleavage egg (left) at 0 concentration EarthTec and an uncleaved egg (right) at 60 concentration of EarthTec.

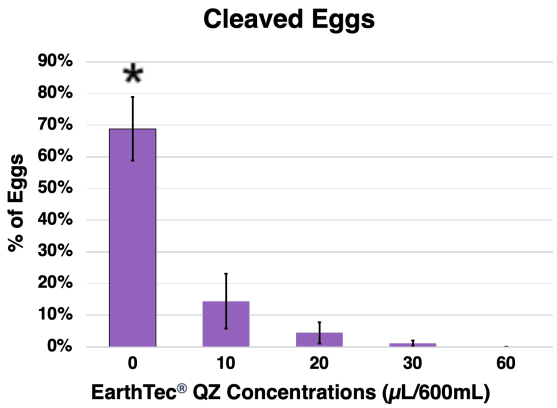


*Figure 10b. Mean percentage of eggs cleaved for three trials after 90 minutes. Bar-SE. *-significant difference ($p<0.05$)*

Similar to time point 5 and 20, some eggs were observed to have broken open. Figure 11 shows an egg at concentration 20 after 90 minutes. The circular shape of the egg is faint and egg contents and sperm appear to be surrounding it.

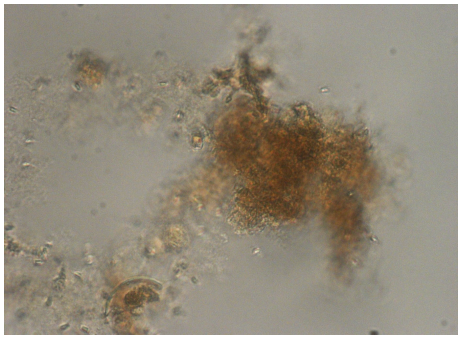


Figure 11. Phase contrast image of a burst egg after 90 minutes.

Additional Observations

In adult mortality trials, blue buildup accumulated on the shells and in the water. At high concentrations, there was brighter buildup. Most of the blue color concentrated at the base of the shell as shown in Figure 12. The solutions also contained specs of blue between solution replacements. Formation of pale blue droplets appeared around the mussels as well. None of these appeared in the control group.

Figure 12. Blue buildup shown on the shell (left) and in the solutions (right).

DISCUSSION

EarthTec QZ has been shown to affect the survival of zebra mussels in previous studies (Watters et al. 2013, Claudi et al. 2014, Lund et al. 2018, Barbour et al. 2018, Hammond & Ferris 2019, Lake-Thompson & Hoffman 2019). The results in the present study shed some light on the effect of EarthTec QZ on adult mortality under controlled laboratory settings and its effects on reproductive viability in a closed system.

Adult Mortality Conclusions

The relationship between mortality and EarthTecQZ was not significant at day six of mortality trials. Removing the one outlier trial demonstrated EarthTecQZ did have a significant effect. This experiment should be repeated to make sure the one trial was just an outlier. However, over time EarthTecQZ was determined to have a significant effect on the survival probability of zebra mussels and the concentration did not matter. By day six there was almost 100% mortality in all concentrations. This demonstrates that the smallest concentration used

could be tested further and potentially implemented to control zebra mussel populations. The 10 µL/600 mL solution has a copper concentration of 0.01 ppm, which is well below the EPA's maximum of 1.3 ppm, so it is classified as acceptable as an additive to water. Further studies could test concentrations below 0.01 ppm to see if they would be as effective in order to limit the amount of EarthTecQZ put into water.

The blue coloring on the mussels showcases the effect of EarthTecQZ once taken up by the zebra mussel. The copper remains in the shells once the mussels die, therefore additional studies could look at the impact of the aggregation on the ecosystem. Accumulation of blue buildup in the solution may pose problems if EarthTecQZ is implemented. The small containers could have allowed for easy buildup, so in an open reservoir or body of water, the same phenomenon may or may not occur. Testing in a larger capacity should be conducted to see if buildup happens and if that has any impact on other organisms.

Adult Mortality with Chlorine Conclusions

Chlorine trials were originally going to be used as a control group since bleach is typically used as a way to kill aquatic organisms, commonly in pools. The low levels of chlorine used in this study were previously shown to kill quagga mussel veligers (LaCroix and Archaryadid, 2011). However, the low chlorine levels did not significantly impact the mortality of adult zebra mussels in the present study. A potential reason why could be due to mature zebra mussels' increased resilience towards external toxins. When exposed to noxious chemicals, bivalves will close their shells to prevent exposure to internal tissues to the toxin. While the tested levels of chlorine can decrease the amount of veligers, thus the amount of adults later on, it could not be used as a way to decrease already formed adult zebra mussel populations. The

concentrations required would be too high according to the EPA to safely put in communities, since the current limit is 4.0 ppm.

Fertilization and Early Development Trials

Overall, the four EarthTecQZ solutions were able to decrease reproductive viability. They did not significantly impact the amount of sperm bound 5 minutes post insemination, also suggesting no acute impact on a sperm's ability to swim towards the egg. Previous studies in our lab showed that higher doses of EarthTec QZ (0.06 ppm Cu^{2+}) showed significantly decreased sperm binding and reduced motility but lower doses (0.01 ppm Cu^{2+}) did not (Martinez, 2020). While copper is known to impact sperm motility (Jecht and Bernstein, 1976, Roblero et al., 1996), the short term exposure of copper experienced by sperm in our study may be insufficient to significantly impact sperm motility needed to reach and bind to the egg.

A sperm's ability to enter and fertilize an egg was impacted by the addition of EarthTecQZ as shown in the 20 minute fertilization time point. However out of the four concentrations, only the 60 concentration significantly decreased the number of fertilized eggs. The number of cleaved eggs was significantly impacted by all copper concentrations. If egg cleaving does not occur, the rest of the development of an embryo will not proceed and the embryo will die. This suggests that EarthTecQZ would be preventing the reproduction of zebra mussels. Future experiments can repeat these treatments on veligers to reinforce this hypothesis since they are further along in the development process. Additionally, trials can be repeated with concentrations below 10 to determine if a smaller concentration could still have affected reproductive viability to minimize the solution used. Another potential study could look at altering the time in which gametes were incubated in the copper solutions. This study incubated

gametes for five minutes, however it may be possible that a longer incubation could have an effect on sperm binding.

When looking at the eggs across all time points, eggs exposed to any of the copper concentrations had abnormal shapes in a number of samples. These observations were never present in the control group. The change in circular shape could potentially be due to the paraformaldehyde, as it fixes the egg in a set position. However, it does not typically break eggs open and since the control groups did not have breakage, paraformaldehyde is unlikely to have caused this. It is possible that the EarthtecQZ disrupted the cell membrane, causing the internal contents to spill out. If this is the case, EarthTecQZ did have an effect on the eggs at all three points through early development and not just sperm. Further research can shed light onto whether this membrane burst is due to the addition of EarthTecQZ.

Impacts on Current Understanding

EarthTecQZ is currently being tested and used in contained bodies of water to decrease zebra mussel infestation. These results identify the 10 µL/ 600 mL, or 0.17 ppm, as effective to cause mortality in adult mussels, as well as decrease reproductive viability. This concentration was the lowest tested and still happened to be effective, therefore would be preferable to use. In addition, it is well below the EPA limit of 1.3 ppm. Using 0.17 ppm limits the amount of copper added into water and can lessen potential impacts on other organisms in the ecosystems. Since artificial pond water can't fully mimic the environment lake water provides due to the presence of other organisms, further testing can be done to determine the breadth of effect this concentration can have within an ecosystem.

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