Investigating chemical treatment options on quagga mussel veligers in static conditions for treating water supplied for agricultural use & determination of water source effect on adult quagga mussels

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Executive Summary

United Water Conservation District (UWCD) has been tasked with ensuring no quagga mussels reach their customers. Quagga mussels were found in Lake Piru, CA in 2013, which is a waterbody owned and managed by UWCD. UWCD delivers surface water from Lake Piru to the Oxnard Forebay using the Santa Clara River as conveyance. Surface water is diverted at the Vern Freeman Diversion where it can be spread at UWCD's groundwater recharge basins or delivered directly to agricultural customers in the Oxnard Plain. Veligers, which are the larval stage of mussels, can be easily carried downstream in the water currents. AECOM drafted a report in 2016 that suggests control alternatives to prevent quagga mussels from entering UWCD infrastructure or at least not entering downstream stakeholder infrastructure.

One of the most cost-effective control alternatives proposed by AECOM is to use a chemical treatment system to kill the quagga mussel veligers at the Freeman Diversion or at the Moss Screen. There is no established population outside of Lake Piru and Piru Creek currently, so veliger testing was the primary focus. UWCD contracted KASF Consulting to perform chemical testing to determine the efficacy on quagga mussel veligers.

A range of concentrations was tested for three chemicals, which are chlorine, potassium permanganate, and a copper product EarthTec QZ. Since the control alternative would require year-round constant treatment, this study used a range of temperatures, 10, 15 and 20 ºC. Flow rate through the system can vary, but short treatment times were ideal with 24 hours as the maximum exposure duration. Veligers were collected from Lake Piru and exposed to a suite of concentrations for each chemical with Lake Piru water as the treatment water. After exposure duration, veligers were rinsed, dyed and evaluated to determine mortality. Water quality measurements were taken at the beginning and end of exposure durations.

This study showed that about 50% mortality could be achieved in 24 hours with any of the three chemicals at their highest concentrations tested (10 mg/L as chlorine, 20 mg/L potassium permanganate, and 16.7 µL/L EarthTec QZ). The chlorine trials resulted in the highest mortality at 10ºC. However, there was no significant difference in mortality observed between the highest concentrations of the chemicals at 24 h. This study showed that chlorine and potassium permanganate did not achieve comparable toxicity results to published data. The concentrations needed to achieve 100% mortality in the published studies resulted in mortality equal to control mortality. Much higher concentrations and longer durations were needed to achieve 40 to 50% mortality. EarthTec QZ also resulted in mortality of veligers at 24 h, but longer exposure durations were needed to obtain higher mortality results. Possible reasons for the difference in results include different water qualities; this study had higher pH (greater than 8.5) compared to other studies. Chlorine's effectiveness decreases as pH increases above 7.0. Other studies with these chemicals have relied on observation of the veligers, which is cumbersome and inaccurate, resulting in studies evaluating a small number of veligers or error in assessing mortality. This study used the fast green stain method to ensure high confidence in assessing mortality, which speeds up the evaluation time allowing for a larger sample size to be assessed.

Water chemistry was within acceptable ranges to support veliger life. Turbidity and chemical oxygen demand of the testing water was very low and did not cause chemical deactivation. The pH during testing was high at 8.6 and chlorine is more toxic at a neutral pH. Suggested prospective testing includes further study of chlorine's toxicity to veligers at different pHs. Another option is to look at the toxicity of flocculant combined with chlorine.

Another control alternative that was suggested by AECOM was to pump groundwater out of the Oxnard Forebay and use it to supply the agricultural customers. UWCD wanted to alternate water sources and deliver nutrient-starved ground water when needed. Adult mussels from Lake Piru were put into containers with surface water from the Freeman Diversion and well water from the Pumping Trough Pipeline Well No. 2 with the control being surface water from Lake Piru. Mussel mortality was high for the two-month duration of the tests. Temperatures were not easily controlled and increased to above 30ºC causing significant mortality. This study showed that adult mussels were intolerant to temperatures greater than 27.5ºC. Water source did not influence mortality. Adult mussels have been shown to survive without food for 166-945 days depending on temperature. Veligers can go without food for up to 15 days. This study showed that including veliger-laden water into pumped groundwater would not control the quagga mussels.

Other alternatives that AECOM proposed included the use of an infiltration gallery. This report did not test the effectiveness of one. Prospective testing could include testing a model of an infiltration gallery built by AECOM or another partner. Investigation into other mechanical methods, such as filters, could be a reasonable solution if technology has advanced in this area from the early 2000s. KASF Consulting would be willing to test the mechanical methods and the chemical combinations discussed. Much of this testing would need to occur in flow-through systems, which could be completed with some modifications at the Lake Piru research trailer.

Introduction

Invasive dreissenid, zebra and quagga, mussels (*Dreissena polymorpha* and *Dreissena bugensis*) arrived in the United States from Europe in the 1980s and quickly spread into many Eastern US waterways, rivers, and lakes. Since 2007, quagga mussels have been present in the lower Colorado River and were introduced via aqueducts into California. In late 2013, quagga mussels were found in Lake Piru in Ventura County, Southern California. This was the first-time quagga mussels were found in California in a lake or reservoir that does not receive Colorado River water.

United Water Conservation District (UWCD) operates and manages many facilities that are supplied with water from Lake Piru. UWCD is considering control measures to limit the spread of quagga mussels from Lake Piru into other water bodies and its infrastructure (AECOM 2016). The Vern Freeman Diversion is the first point that quagga mussels contact UWCD infrastructure outside of Lake Piru which diverts and delivers surfacewater along the Santa Clara River directly to agricultural users in the Oxnard Plain or indirectly through conjunctive use operations as wholesale drinking water to municipal and industrial users. UWCD has been tasked with ensuring no mussels reach these users.

One of the options presented to UWCD is chemical treatment to maintain and limit the quagga mussel population in the facilities. The four chemicals proposed to conduct treatments were chlorine, EarthTec QZ (active ingredient: copper sulfate pentahydrate), potassium chloride and potassium permanganate. These chemicals were highlighted because of their application in drinking water treatment or success in veliger eradication. An infiltration gallery and the use of groundwater in the supply line were other non-chemical options.

Chemicals containing copper have historically been used for treating algae from surface water. Chlorine and potassium permanganate were used in drinking water disinfection. Potassium chloride was typically used for water softening, but has been successful in dreissenid mussel treatments and not harmful to other organisms in the water body treated. Success of chemical treatments depends on temperature stability, maintaining concentration through flow events, and water chemistry influences (Moffitt et al. 2016; Stockton-Fiti and Moffitt 2016; Luoma et al. 2018). Chemical treatment is possible if maintained during storm events and monitoring of dosage and mortality of mussels is routinely conducted.

The water quality and parameters at the Freeman Diversion vary throughout the year. Data supplied to KASF Consulting from UWCD of Vern Freeman Diversion water testing show that temperature ranges from 3 to 25°C with an average 16°C throughout the year. Conductivity is high in the basin at about 1 mS/cm compared to <0.5 mS/cm in the Great Lakes region where many of the chemical studies have been conducted on dreissenid mussels. As Moffitt et al. (2016) showed sodium concentrations influence the concentration of potassium control chemicals; as sodium increases the amount of potassium needed to be effective increases (Stockton-Fiti and Moffitt 2016). Vern Freeman Diversion surface water has concentrations of sodium ranging from 33 to 200 mg/L with an average of 110 mg/L. The average sodium concentration is high and is comparable to sodium levels found in the Colorado River, where

potassium treatments were not effective (Moffitt et al. 2016). Total organic carbons or another parameter indicating concentration of organic particles may increase the concentrations of chemicals easily deactivated by organics such as chlorine, potassium permanganate, and EarthTec QZ.

Chlorine is one of the oldest mussel treatment options available, but has been criticized for its toxicity to all organisms and potential toxic by-products (Sprecher and Getsinger 2000). Concentrations of chlorine from sodium hypochlorite at 0.5 mg/L killed 100% of veligers within 2 hours in a static treatment and produced 83% mortality in flow-through applications at variable temperatures (AWWA 1997; Stevenson 1997). Chlorine can be easily neutralized, is inexpensive, and easily applied; however, chlorine involves hazards such as requiring special handling because of its corrosive properties and forms trihalomethanes (Sprecher and Getsinger 2000). Veliger and adult mussels can sense chlorine and close up until treatment concentration is not toxic or treatment stops (Van Benschoten et al. 1993; Sprecher and Getsinger 2000; Mackie and Claudi 2010).

EarthTec QZ (active ingredient: copper sulfate pentahydrate) is a recent chemical to come to market and has had success in open water and in raw water intake applications for treatment of mussels and algae. The active ingredient is copper in the cupric ion form, which is the mostly biologically available form of copper. Tests on adult mussels show low doses, <1.0 mg/L, with an exposure duration of 96 h were effective (Claudi et al. 2013). Watters et al. (2013) found that 100% veliger mortality occurred in 30 min at 3 ppm. EarthTec QZ is EPA registered as a molluscicide for prevention and control of quagga and zebra mussels (EPA 2015) and is registered in more than 26 states that have invasive mussels. EarthTec QZ's effectiveness is limited by temperature (Luoma et al. 2018). Copper is regulated by the Division of Drinking Water in California and has a secondary maximum containment level of 1.0 mg/L (State of California 2018). This will regulate the maximum dose of copper to be tested. Another limit to the highest concentration used will be limits on agricultural products as copper is considered toxic to plants (rice, wheat, corn, barley, oats, rye, sorghum, millet, soybeans, beans, peas, spinach, and citrus seedlings) at concentrations of 0.1 to 1.0 mg/L copper (Bureau of Reclamation 2005). Copper is a micronutrient for plants and animals and is utilized which allows for limited use of copper products in the environment.

Potassium chloride was discovered to be toxic to mussels in early 1990s. It was considered a more selective toxicant, and was used in large-scale open water treatments with success (Luoma et al. 2018). Moffitt et al. (2016) showed that using KCl at 960 mg/L would kill 100% of the quagga mussel veligers in low sodium containing waters in 12 hours. In very low conductivity water with low sodium concentration, the calculated LT50 (mean time to 50% mortality) was 2.7 hours. The time to LT50 increased with increased conductivity and concentrations of sodium in the water to the point where no mortality was observed in high conductivity waters (1.08 mS/cm) within a 24-hour exposure period. Stockton-Fiti and Claudi (2017) used 8.5 g/L of potassium chloride to obtain 60% mortality with an exposure time of three hours in Colorado River water with conductivity of 1.01 mS/cm. Potassium chloride's toxicity is temperature dependent (Luoma et al. 2018). High concentrations of potassium chloride was expected to be successful

and UWCD determine that concentrations greater than 1 g/L were not feasible, so no testing was conducted on this chemical.

Potassium permanganate is a common drinking water treatment with variable success in controlling dreissenid mussels. Concentrations of 2.5 mg/L were used in static conditions with up to 36% mortality in 1 h, but longer exposure duration achieved less mortality (AWWA 1997). Coyle et al. (2014) showed that 8 mg/L potassium permanganate resulted in 60% mortality in 3 hours. High concentrations of potassium permanganate cause the water to turn pink and can be a aesthetic issue for drinking water suppliers. Though not as well tested as potassium chloride, potassium permanganate's toxicity may also be dependent on sodium concentrations and temperature.

Implementing mechanical removal tools, such as a sand infiltration gallery or drum filter, for the water being diverted into the Freeman Diversion is an option. UWCD also has some ability to control the type of water coming into part of their systems and has recently relied on groundwater to operate their Pumping Trough Pipeline (PTP). If the nutrient-starved groundwater will not support mussel growth and survival, then this could be a possible way to control the mussels.

Some chemicals have been studied more than others to control dreissenid mussels; however, there are different water qualities across the nation that affect the toxicity of the chemicals. It is important to understand how these chemicals work given the particulars of the treated system (Luoma et al. 2018). This study investigated three chemicals (chlorine, EarthTec QZ, and potassium permanganate) to determine the effectiveness of a range of concentrations within a range of temperatures. This report is meant to guide UWCD in determining if a chemical treatment option can be successful given their water quality and if the water quality will support adult mussels. Additionally, this study looks at next steps to discuss possible flow through treatments with chemicals or doing additional studies with mechanical options such as filters or infiltration galleries.

Project Methods

Chemical Control Study on Veligers with Chlorine, EarthTec QZ, and Potassium Permanganate

Quagga mussel veligers were collected from Lake Piru with a 50 µm plankton tow net with the assistance of United Water Conservation District (UWCD) staff from June to July 2019. The collection was passed through a 500 µm filter onto a 63 µm filter and back flushed into a beaker with ~30-200 mL of 10 µm filtered water collected from Lake Piru dam to obtain a concentrated sample of veligers. Veliger concentrate was assessed for density of live (physically moving or tissue movement of veliger), dead (cracked shells or degraded tissue), or empty shell (with no tissue and shell remaining). If veliger concentrate contained >5% dead to live veliger density, the plankton collection was not used. The veliger concentrate was divided into testing beakers to achieve approximately 100 to 200 live veligers per beaker, ranging in size from D-shaped (>63

µm) to pediveligers (< 300 µm) per replicate in filtered lake water. This division was done within 1 h after density evaluation. Additional 10 µm filtered water was added to ensure there was 50 mL total in each beaker. Three replicate beakers were used to evaluate chemical toxicant and controls (i.e. no chemical toxicant) for each treatment temperature, concentration, and exposure duration combination. When 450 mL of the chemical stock solution was added to the beaker to obtain a final treatment concentration this started the exposure duration.

The chlorine concentrations were 2, 5 and 10 mg/L as Cl_2 made from 5% w/v sodium hypochlorite (LabChem lot# H249-14). EarthTec QZ concentrations were 3, 10 and 16.7 ppm (µL/L), equivalent to 0.18, 0.6 and 1.002 mg/L as copper, diluted from stock concentration provided by EarthScience Labs, Inc. (lot# 181710). Potassium permanganate concentrations tested were 8, 15, and 20 mg/L made from analytical grade product (Sigma-Aldrich lot# MKCG6941). A 1.1X concentrated stock solution was made for each concentration. Stock solutions were held at testing temperature. Chlorine, copper and manganese concentrations were measured with a Hach DR900 spectrophotometer to ensure that concentrations were correct.

Each product was tested at 3 temperatures, 10, 15, and 20°C in lake water collected from Lake Piru. Temperature was controlled by placing the beakers in a water bath using a chiller or heater to control the water temperatures. Temperature was monitored with water quality probes and logged with a temperature logger.

Exposure durations included 1, 3, 6, and 24 h; however, studies with chlorine went 7 h instead of 6 h. Trials with each chemical, temperature and treatment duration had staggered start times. A control (no chemical) was paired with each chemical and exposure time for each temperature completed.

To end each replicate, the contents of each beaker were poured onto a 50 µm filter and the beaker was rinsed three times with filtered lake water onto the filter for analysis. Each beaker's contents were analyzed with the fast green stain method for determining live and dead in the samples (Stockton-Fiti and Claudi 2017). An aqueous solution of 0.4% fast green FCF (Sigma-Aldrich lot# MKCG7515) was prepared with filtered lake water. The sample concentrate on the 50 µm filter was placed into a dish with <5 mL fast green stain and held for 20 min. The contents were then rinsed with filtered lake water until dye was not visibly present and then back flushed from the filter into a recovery beaker with <5 mL of filtered lake water. A 2 mL sample of veliger concentrate was removed from each recovery beaker with a disposable pipet and visually evaluated using a gridded Sedgewick-Rafter counting cell on a compound microscope (total magnification of 40 and 100X). Live veligers were defined as minimally stained with mantle intact, dead veligers had stained mantles, and open empty shells were not stained and had no mantle present. Approximately 100 veligers were assessed per beaker.

Dissolved oxygen, conductivity, pH and temperature were measured with a Hach HQ40d with LDO101, CDC401, and PHC705 (PHC201) probes (Hach, Loveland, CO). These parameters were recorded at initial start of experiment and at the end of the exposure time. Free and total chorine, free and total copper or manganese concentration was measured at the start and end of each exposure with the Hach DR900 spectrophotometer.

During the final check prior to ending an exposure duration, the pH probe broke in the EarthTec QZ 10 ppm 6 h replicate 2 beaker at 15ºC. Mortality was considerably higher than the other two reps 42% instead of 13%. Because of this accident, additional testing with 50 mg/L and 400 mg/L potassium chloride (Macron Fine Chemicals lot# 66919) added to the stock solutions was conducted with EarthTec QZ at 20ºC for 3, 10 and 16.7 ppm (µL/L) with a 1 hour exposure duration. The stock solutions were prepared and then the beakers containing veligers were dosed with the elevated potassium chloride stock solutions. Same procedures were followed as the previous tests.

Lake Piru water used in the treatments was collected and sent to Fruit Grower Laboratory, Santa Paula, California for analysis of chemical oxygen demand and turbidity. Nine samples were collected, three each week, over the three weeks of testing veligers. Approximately half the samples had veliger concentrate added back into the filtered lake water.

Adult mussel testing to determine the difference in survival with different water sources

Testing vessels were 30 L plastic insulated coolers with an aquarium pump (Quiet One Lifegard Fountain Pump Model 1200, 296-gallon per hour) that recirculated water from the back to the front of the cooler with 10 ft of 5/8 inch clear vinyl tubing. The coolers were designed to recirculate water to keep dissolved oxygen high and ammonia concentrations low.

Water was collected from Freeman Diversion (surface) and PTP (Pumping Trough Pipeline) well 2 (well) on March 28 and April 1. Additional surface water was collected May 30. The collected water was stored in 55-gal barrel drums in the maintenance shop at Lake Piru dam. Lake water was from a tap at the outlet of Lake Piru and was pumped as needed for water changes. Complete water changes occurred if the dissolved oxygen was below 5 mg/L or the ammonia was higher than 0.5 mg/L. Complete water changes occurred every 7 to 14 days. Tests were conducted at ambient temperature.

Round 1

Adult mussels were collected from Lake Piru, CA from December 2018 to March 2019. Testing was initiated on March 29, 2019. Mussels were gently separated and condition assessed. Mussels were divided up into 5 cups per cooler with 4 cups containing 33 mussels and 15 mussels were in the last cup for a total of 180 mussels per replicate. Cup A mussels were measured prior to being put into the cooler and measured when determined to be dead. Three coolers were filled with 20 L of surface water from the Freeman Diversion and three coolers were filled with 20L of well water. The study ended after 7 weeks.

Round 2

Adult mussels were collected from Lake Piru, CA from April to May 2019. Testing was initiated on May 23, 2019. Mussels were gently separated and condition assessed. Mussels were divided up into 4 cups per cooler with 35 mussels for a total of 140 mussels per replicate. Cup A mussels

were measured prior to being put into the cooler and when determined to be dead. Three coolers were filled with 20 L of surface water, three coolers were filled with 20L of well water, and three coolers were filled with 20L of Lake Piru (lake) water, see Appendix A for water quality information on Freeman Diversion and Lake Piru water. This data shows that the water quality is similar chemically to support quagga mussel life and development. The study ended after 2 months.

Mussels were checked for mortality weekly and dead mussels were disposed. Dissolved oxygen, conductivity, pH and temperature were measured with a Hach HQ40d with LDO101, CDC401, and PHC705 (PHC201) probes (Hach, Loveland, CO). Ammonia was measured using the Hach Nitrogen, Ammonia Test Kit (model NI-SA). Water chemistry checks were completed every three to four days.

Statistical Analysis

Mortality was calculated using the amount of dead over the sum of live and dead for each replicate of each treatment. For veligers, the empty shells were not included in the assessment of dead veligers due to few numbers and being present prior to testing. Control mortality at 1 h showed that background mortality was less than 4%, with an average control mortality of 2.2±1.6%. The mortality was not adjusted for control mortality, as this is relatively low mortality when observing veligers in static conditions. Mortality of the different treatment concentrations were compared to the corresponding control mortality using Welch's t-test in Excel, which assumes unequal variance in the samples.

The water quality measures were summarized to provide a mean temperature, dissolved oxygen, specific conductance, and pH. Conductivity was converted to specific conductance to compare across temperature ranges. The formula to convert conductivity was:

$$SC = \frac{C}{1+(r(T-25))},$$

where SC=specific conductance, C=conductivity; T=temperature is in Celsius and r is the temperature correction coefficient for the sample, 0.0191 (Carlson 2015).

A general linear model was used to evaluate the relationships between temperatures, concentrations, or exposure times for mortality and each of the measured water quality parameters to determine differences. The GLM procedure was conducted in R 3.1.3 (R Development Core Team, 2015) with package *lme4* (Bates et al. 2014). Tukey's HSD was used to determine how the variables related to each other if significant differences were found using package *multcompView* (Graves et al. 2012).

General linear modeling in Excel was used to determine the expected date of mortality of the adult mussels in the different source waters. A liner trendline was found and 100% mortality was calculated to determine the date for each source water.

Results

Veliger Static Study

Chlorine Trials

Veligers were susceptible to chlorine at high concentrations with a peak of 52% mortality observed at 24 h in the 10 mg/L chlorine at 10ºC (Table 1; Figure 1). The high concentration resulted in the highest mortality at all temperatures. There was none to low mortality (<20%) in the lower concentrations (Table 1). As concentration and duration increased, mortality increased for each temperature. For each temperature trial, both concentration and duration were significant variables in predicting mortality. Only the 10 mg/L chlorine concentration had significantly different mortality than the control mortality ($F(3):19.82$; p-value:<0.001). There was a linear relationship of duration for the 10 mg/L chlorine treatment for mortality (Figure 1). Temperature was not a significant variable at 24 h in the 10 mg/L trials ($F(2):1.899$; p-value: 0.23). In this study, temperature was not an important variable in predicting mortality.

During testing, temperatures stayed consistent, within 1.0ºC of testing temperature. At the start of the 10ºC trials, the temperatures were about 14ºC, but obtained 10ºC by 3 h. This higher than expected temperature could have impacted results, but were consistent with the 3h mortality results (Figure 1). The 15 and 20ºC tests were at the expected temperature the entire duration. Average dissolved oxygen for all beakers over all testing concentrations and durations was 9.2 mg/L. There was only a slight drop in dissolved oxygen levels to 6.4 mg/L in the control 24 h at 20ºC, but this had no effect on mortality (Table 1). The pH was consistent over time and did not fluctuate; the average pH was 8.6.

Table 1. Mean mortality with standard deviation of chlorine trials tested by temperature. Treatment mortality that was significantly different from the control mortality are represented by *p<0.05, **p<0.01, ***p<0.001.

Temp. (ºC)	Cl_2 Conc. (mg/L)	1 h	3 h	7 h	24 h
10	0	1.9±1.2%	1.9±0.9%	1.8±0.5%	0.6±0.5%
	2	0.8±0.9%	0.5±0.9%	0.9±0.1%	0.6±1.0%
	5	3.6±2.9%	1.2±1.0%	2.6±1.5%	10.0±3.2%*
	10	2.3±0.4%	2.7±1.6%	8.5±1.2%**	51.7±11.7%*
15	0	1.7±1.6%	6.6±2.6%	4.7±3.5%	5.4±2.9%
	2	3.3±3.1%	7.2±1.0%	7.9±5.6%	6.4±4.1%
	5	4.3±2.2%	8.5±2.1%	9.4±6.8%	12.2±7.5%
	10	6.1±2.8%	10.8±4.6%	30.2±6.1%**	34.3±14.9%
20	0	3.9±3.1%	4.7±1.9%	6.4±5.7%	3.3±2.1%
	2	5.5±3.5%	1.8±0.5%	5.9±3.2%	5.8±0.8%
	5	6.2±0.2%	11.0±6.9%	6.4±3.3%	18.8±4.6%*

10	4.1±1.8%	10.2±2.4%*	21.1±10.4%	37.9±6.3%**

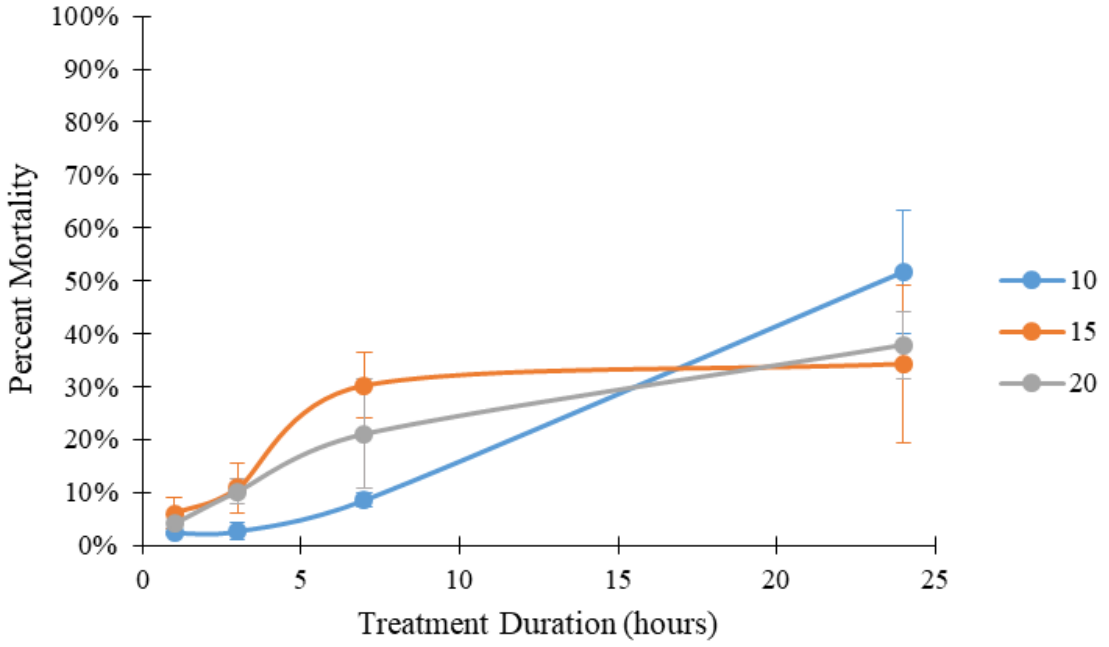

Figure 1. Mortality with standard deviation bars for 10 mg/L chlorine at each tested temperature.

Specific conductance did differ between temperatures, indicating that it differed over testing days. During the 10ºC tests, the specific conductance was approximately 1.1 mS/cm at the beginning of testing and increased to 1.25 mS/cm over 24 h (Table 2). At this temperature, the specific conductance increased with concentration (F(3):5.167; p-value: 0.004), with higher concentrations having higher conductivities. Specific conductance was different over time (F(3):13.61; p-value:<0.001), but Tukey's HSD showed that 0 and 1 h were similar and 3, 7 and 24 h were similar. At 15ºC the specific conductance increased significantly with increased concentration (F(3): 38.2; p-value:<0.001). Duration was not a significant variable in the 15℃ tests. In the 20ºC specific conductance did not increase significantly with duration (F(3):0.275; p-value:0.843), but did increase with concentration (F(3):194.54; p-value: <0.001) (Table 2).

The chlorine stock solutions were made the morning of treatment. Total and free chlorine values were measured to determine the concentration of chlorine. The calculated amount of chlorine was added to the stock solution and then tested. On many occasions the concentration was lower than expected when tested to determine final concentrations. Additional chlorine was added until average total chlorine values were within 0.2 mg/L of expected stock solution concentration (Table 3). On other occasions the concentrations were higher than expected, up to 3 L were removed from the stock solution and replaced with non-chlorinated water. There were many issues with chlorine concentrations. On the last round, 20ºC, concentrations were left high to obtain higher treatment concentrations within the trial.

Table 2. Average specific conductance (µS/cm) readings for the chlorine trials tested by temperature.

Temperature	Concentration	0 h	1 h	3 h	7 h	24 h
10ºC	0 mg/L	1134	1167	1228	1214	1251
	2 mg/L	1132	1184	1239	1235	1254
	5 mg/L	1185	1224	1235	1282	1248
	10 mg/L	1176	1234	1244	1284	1244
15ºC	0 mg/L	1063	1064	1080	1086	1094
	2 mg/L	1073	1075	1092	1100	1097
	5 mg/L	1089	1109	1099	1096	1107
	10 mg/L	985	1123	1114	1110	1124
20ºC	0 mg/L	871	894	893	898	909
	2 mg/L	891	907	906	910	921
	5 mg/L	917	917	915	900	918
	10 mg/L	972	967	967	965	972

Actual testing concentrations were lower than desired (Table 4). Total and free chlorine concentrations were not the same, as free chlorine concentrations were generally lower than total chlorine concentrations. In general, the standard deviation between replicates was less than 0.5 mg/L, but a few of the trials had larger standard deviations. The average total chlorine concentration, combining the three temperature treatments for each testing concentration, was 2 mg/L=1.13 mg/L Cl_2, 5 mg/L=3.38 mg/L Cl_2, and 10 mg/L=7.93 mg/L Cl_2.

Chlorine concentration decreased in the trials over time, but in some instances increased at 24 h (Table 4). The most notable example of this increase was in the 10ºC trials. For example, in the 10 ℃ trials, the chlorine concentrations quickly decreased within the first few hours to levels around 1.0 mg/L, but increased when read at the 24 h. Total chlorine concentrations were greater than 1.0 mg/L after 3 h in the 10 mg/L trials and were less variable. In the 15 and 20℃ tests the chlorine decayed over time and did not increase to the higher levels as occurred in the 10℃ tests. The 10℃ chlorine trial was run on the second day of testing, after completing the chlorine 15℃ trial, which eliminated user or equipment malfunction and mistakes. Low temperature and high pH could have influenced the reactions of the chlorine molecules with water and the organic matter.

Table 3. Process of adding 5% sodium hypochlorite solution to make chlorine stock solution, where total chlorine reading was evaluated and more chemical was added to obtain correct readings. If concentrations were too high, up to 3L were removed and equal amount replaced with non-chlorinated water (ex.-3LR3). If too much was removed, saved stock solution (SS) was added back in after removing equal volume. Free chlorine levels were measured once the stock concentration was at correct concentration. Gray highlighted cells are the final total chlorine readings prior to use in testing.

Temp	Chlorine	Added (µL)	Total	Added (µL)	Total	Added (µL)	Total	Free
15ºC	2 mg/L	320	2.1,1.8,1.8	+20	1.6,2.1	20	2.0,2.5	0.3,0.0
	5 mg/L	815	7.9,7.5,7.7	-1LR1	5.5			0.8,0.0
	10 mg/L	1615	18,19.2,21.6	- 3LR3	8.9,9.1	+200mLSS	10.8,11.0	
10ºC	2 mg/L	320	2.7,2.7					2.3,2.3
	5 mg/L	815	7.4,7.5	-1LR1	6.0,5.8			5.5,5.9
	10 mg/L	1615	8.4,8.0	+200,+100,+100+200 +200+100+200	10.6, 22	-1LR1, -2LR2, -1.5LR1.5	10.3,7.9	10.2, 10.0
20ºC	2 mg/L	320	1.2,2.0	+20	1.1,1.1	+120	2.1,2.1	2.3,2.3
	5 mg/L	814	4.8,5.0	+50	5.4,5.2	20	5.5,5.4	6.0,6.0
	10 mg/L	1620	7.2,6.4	+100	14.7,13.2			16.2,17.1

Table 4. Average total and free chlorine concentrations with standard deviations over time for the trials. The 0 hour was within 10 min of pouring the stock solution in to the veliger containing water in the test beaker.

		Total Chlorine (mg/L)					**Free Chlorine (mg/L)**				
Temp. (ºC)	**Conc. (mg/L)**	**0 h**	**1 h**	**3 h**	**7 h**	**24 h**	**0 h**	**1 h**	**3 h**	**7 h**	**24 h**
10	0	1.2±0.6	0.5±0.1	0.9±0.6	1.3±0.6	5.2±2.7	0.6±0.4	2.4±0.9	0.1±0.1	1.2±0.5	4.8±2.2
	2	0.8±0.2	1.2±1.0	2.2±0.9	1.3±0.4	6.8±0.6	0.5±0.2	1.1±0.3	0.9±0.4	3.2±2.0	4.2±0.4
	5	3.5±0.3	3.4±0.2	3.1±0.5	3.6±0.3	5.5±0.9	1.7±0.6	1.2±0.3	1.6±0.1	1.9±0.9	2.0±0.7
	10	6.6±0.3	5.7±0.2	4.2±1.1	4.8±0.1	2.9±0.4	4.4±0.6	2.4±0.8	1.5±0.8	4.2±2.0	1.3±0.5
15	0	1.2±0.3	0.9±0.2	0.8±0.2	0.3±0.3	1.3±0.8	0.6±0.4	0.3±0.2	0.9±0.2	0.0±0.0	0.7±0.6
	2	2.2±0.3	1.6±0.1	1.5±0.2	0.3±0.3	0.8±0.4	1.2±0.1	0.8±0.3	0.7±0.4	0.4±0.8	0.6±0.2
	5	4.3±0.2	3.9±0.3	3.0±0.5	1.1±0.3	0.8±0.1	1.6±0.4	1.5±0.2	1.6±0.7	0.2±0.2	1.4±0.4
	10	7.5±0.4	6.2±0.2	4.3±0.2	1.8±0.5	2.9±0.3	4.2±1.4	3.4±1.3	1.6±1.1	1.2±1.5	1.3±0.3
20	0	0.1±0.1	0.5±0.0	0.2±0.0	0.3±0.1	0.1±0.0	0.2±0.1	0.6±0.1	0.2±0.0	0.2±0.0	0.3±0.4
	2	0.4±0.2	0.9±0.1	0.3±0.1	0.4±0.0	0.2±0.0	0.4±0.4	1.1±0.1	0.6±0.1	0.6±0.0	0.2±0.0
	5	2.4±0.4	1.0±0.3	0.7±0.1	0.6±0.2	0.3±0.1	3.3±0.1	2.0±0.1	1.4±0.1	1.0±0.1	0.4±0.1
	10	9.7±0.5	7.0±0.2	2.4±0.2	1.4±0.4	0.6±0.0	11.4±0.3	8.9±0.4	4.1±0.3	3.3±0.3	1.4±0.1

Potassium Permanganate Trials

Veligers were susceptible to potassium permanganate. The highest mortality observed was 40.8% after a 24 h exposure to 20 mg/L $KMnO_4$ at 20ºC (Table 5). Overall, mortality at 20ºC was significantly higher than the lower temperatures (F(2):30.87; p-value:<0.001). At 10ºC, mortality in the 8 and 15 mg/L trials were not significantly different from control, but 20 mg/L was significantly different (F(3):5.74; p-value: 0.002). As duration increased, mortality increased significantly in the 10ºC trials (F(3):5.55; p-value: 0.003). In the 15ºC trials, the mortality increased significantly with concentration (F(3):13.90; p-value:<0.001). However, mortality at 8 mg/L was not significantly different from control mortality (Table 5), but mortality at 15 and 20 mg/L were significantly different from control mortality. Exposure time was not a significant variable in the 15ºC trials (F(3):1.64; p-value: 0.193). At 20ºC, mortality significantly increased with concentration (F(3):51.23; p-value: <0.001). The 8 mg/L treatment mortality at 20ºC was significantly different from control, but the 15 and 20 mg/L treatment mortality were not significantly different from each other (Table 5). Exposure duration was not a strong variable in determining mortality in the 20ºC trials (F(3):0.7963; p-value: 0.503).

At all temperatures, the 24 h 20 mg/L potassium permanganate trials resulted in the highest mortality (Table 5, Figure 2). The mortality in the 15ºC trial at 24 h was significantly different from the 10 and 15ºC trials, which were not significantly different from each other (F(2):18.273; p-value: 0.003). Mortality did not increase with increasing temperature.

Table 5. Mean mortality with standard deviation of potassium permanganate ($KMnO_4$) trials tested by temperature. Treatment mortality that was significantly different from the control mortality are represented by *p<0.05, **p<0.01, ***p<0.001.

Temp. (ºC)	$KMnO_4$ Conc. (mg/L)	1 h	3 h	6 h	24 h
10	0	1.2±1.1%	1.1±1.1%	1.1±1.0%	1.2±0.6%
	8	1.7±0.9%	1.8±0.9%	1.8±1.5%	2.6±1.4%
	15	1.7±0.2%	7.7±6.8%	6.1±0.6%**	17.1±3.0%**
	20	3.0±0.6%	3.8±1.3%	9.1±6.0%	34.1±8.5%*
15	0	1.8±0.9%	1.3±0.7%	1.0±1.0%	0.6±1.0%
	8	5.7±2.2%	3.2±2.9%	0.9±0.0%	5.8±4.6%
	15	5.8±1.7%*	3.9±1.7%	2.9±1.0%	5.4±0.6%**
	20	4.7±1.6%	6.4±1.2%**	9.5±2.8%*	15.1±3.5%*
20	0	1.6±1.4%	2.8±1.7%	1.9±0.2%	0.9±0.1%
	8	4.5±0.4%	11.2±2.7%*	13.3±3.5%*	16.1±1.7%**
	15	27.5±4.3%**	30.5±10.8%*	25.4±5.8%*	30.6±6.8%*
	20	24.7±7.5%*	30.0±2.9%***	25.5±5.2%*	40.8±1.8%***

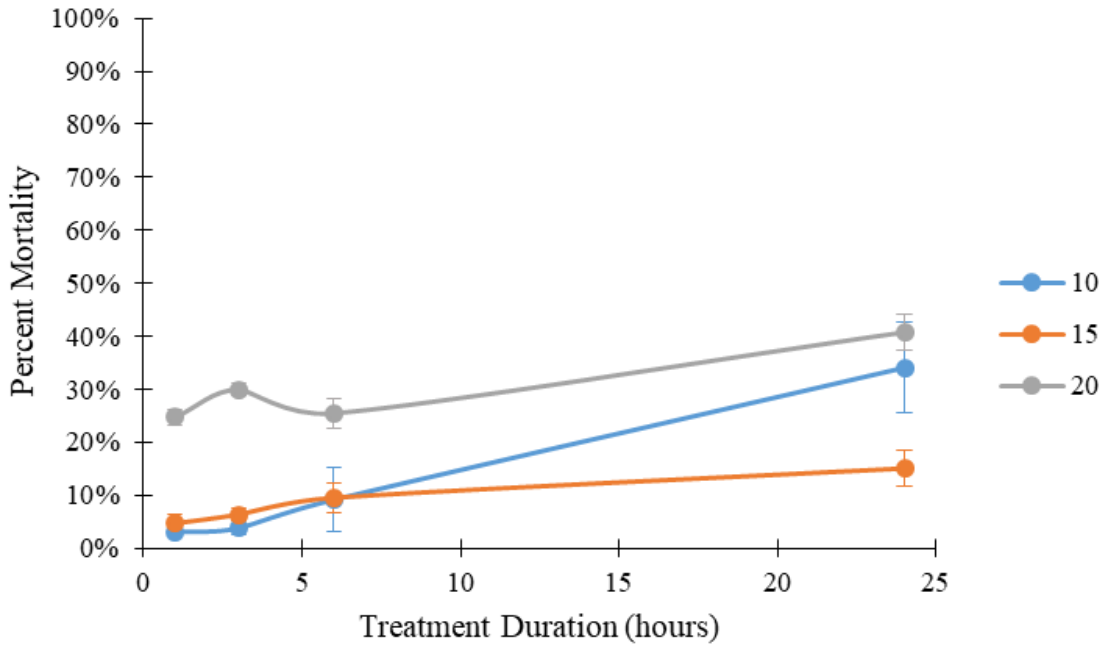

Figure 2. Mortality with standard deviation bars for 20 mg/L potassium permanganate at each tested temperature.

During testing, temperatures stayed consistent, within 1.0ºC of testing temperature. At the start of the 10ºC trials, the temperatures were about 12ºC, but obtained 10ºC at 1 h. The 15 and 20ºC tests were at temperature the entire duration. Average dissolved oxygen for all beakers over all testing concentrations and durations was 9.4 mg/L. There was only a slight drop in dissolved oxygen levels to 6.3 mg/L in the control 24 h at 20ºC, but this had no effect on mortality (Table 5). The pH was consistent over time and did not fluctuate; the average pH was 8.27.

Specific conductance was different between temperatures, indicating that it differed over testing days. During the 10ºC tests, the specific conductance was approximately 1.2 mS/cm at the beginning of testing and did not increase over 24 h (Table 6). At this temperature, the specific conductance did not increase with concentration. At 15ºC the specific conductance was 1.05 mS/cm and did not increase over time or with concentration. The 20ºC specific conductance was 0.9 mS/cm and also did not increase with duration or concentration (Table 6).

Table 6. Average specific conductance (µS/cm) readings for the potassium permanganate trials tested by temperature.

Temperature	Concentration	0 h	1 h	3 h	6 h	24 h
10ºC	0 mg/L	1152.7	1211.0	1214.1	1173.9	1220.6
	8 mg/L	1140.8	1215.0	1218.4	1185.8	1217.5
	15 mg/L	1193.3	1225.0	1207.6	1207.1	1167.2
	20 mg/L	1197.5	1230.7	1207.3	1211.1	1167.5
15ºC	0 mg/L	1034.7	1057.5	1052.2	1061.3	1057.4
	8 mg/L	1045.5	1066.6	1051.0	1068.3	1051.0
	15 mg/L	1051.5	1074.1	1070.2	1065.3	1064.4
	20 mg/L	1061.2	1078.9	1077.3	1071.5	1072.7
20ºC	0 mg/L	865.2	874.4	876.8	875.0	889.4
	8 mg/L	903.3	899.7	904.4	907.3	909.5
	15 mg/L	908.6	894.9	897.0	909.7	907.9
	20 mg/L	909.2	897.3	897.3	903.1	905.0

The 10 and 20ºC potassium permanganate stock solutions were made the evening before treatment and the 15ºC stock solutions were made the morning of testing. Manganese values were measured to determine the concentration of potassium permanganate. The calculated amount of potassium permanganate was added to the stock solution and then tested; many times the concentration was lower than expected (Table 7). The desired value of manganese in the stock solution was the concentration multiplied by 1.1 (for the 1.1X stock solution) multiplied by 0.348 (the proportion of manganese in the chemical =54.938/158.034 g/mol). Additional potassium permanganate was added until average manganese values were within 0.2 mg/L of needed stock solution. When concentrations were higher than expected, up to 1 L was removed from the stock solution and replaced with control water. There were many issues with potassium permanganate concentrations (Table 7).

Table 7. Process of adding granular potassium permanganate to make a 1.1X potassium permanganate stock solution, where total manganese reading was evaluated. When concentrations were too high up to 1L was removed and equal amount replaced with control water (ex.-1LR1). If concentration was too low, stock solution was removed, and saved stock solution from the 20 mg/L (20SS) was added back in after removing equal volume. Additional stock solution was made at 0.04% and added to stock solution.

Temp. (ºC)	Conc. (mg/L)	Added (g)	Mn (-1d)	Mn (0d)	Adjustments	Final Mn
10	8 3.01 Mn	0.05	2.0,2.1	1.8,1.8	+.02,-.5LR.5L15SS, -.25LR.25L20SS	3.2,2.9
	15 5.74 Mn	0.1	4.9,5.4	4.5,4.5	+.02g,-.5LR.5L	5.7,5.5
	20 7.66 Mn	0.13	6.9,6.9	6.3,6.4	+.02g,-.25LR.25,-1LR1LCW, -.5LR.5LP20SS	7.4,7.5
15	8 3.01 Mn	0.05		4.4,4.2	-.5LR.5LCW,-.5LR.5LCW, -.25LR.25LCW, -.5LR.5LCW	2.9,3.3
	15 5.74 Mn	0.1		6.2,6.2	-.5LR.5LCW,-.25LR.25L20SS	5.5,5.7
	20 7.66 Mn	0.13		10.0,9.7	-1LR1LCW	7.5,7.6
20	8 3.01 Mn	0.06	5.0,4.8	4.7	-1LR1LCW,-1LR1LCW, -1LR1LCW	3.1, 2.9
	15 5.74 Mn	0.12	8.8,8.8	8.7	-1LR1LCW, -1LR1LCW, -1LR1LCW,-0.25LR0.25L20SS, -0.2LR0.2L20SS, -.5LR.5L20SS,+1mL0.04%SS	5.6,5.6
	20 7.66 Mn	0.15	10.7, 10.8	13.1	-1LR1LCW,-1LR1LCW, -0.5LR0.5LCW,- 0.25LR0.25L20SS, -0.2LR0.2L20SS,+10mL0.04%SS	7.6,7.4

Manganese concentrations decreased over time in the trials (Table 8). The amount of manganese in the control did not increase. At the beginning of the trial, manganese concentrations were high in the 10ºC and 15ºC trials, but lower in the 20ºC trials. This did not affect mortality as the 20ºC trials had the highest mortality (Figure 2). In general, the standard deviation between replicates was less than 0.5; one beaker in the 15ºC trials at 8 mg/L 3 h duration had a high manganese reading, but it did not influence mortality. The average total potassium permanganate concentration, combining the three temperature treatments for each testing concentration was 8 mg/L=9.3 mg/L $KMnO_4$, 15 mg/L=15.1 mg/L $KMnO_4$, and 20 mg/L=20.4 mg/L $KMnO_4$.

Table 8. Average manganese concentration with standard deviations over time for the trials. The 0 hour was within 10 min of pouring the stock solution into the veliger containing water in the test beaker.

Temperature	$KMnO_4$ Concentration (mg/L)	0 h	1 h	3 h	6 h	24 h
10ºC	0 (0 Mn)	0.8±0.1	0.7±0.2	0.5±0.3	0.7±0.1	0.6±0.2
	8 (2.8 Mn)	3.6±0.2	3.3±0.2	3.0±0.3	2.6±0.2	2.9±0.4
	15 (5.2 Mn)	5.9±0.3	5.3±0.2	5.2±0.3	4.8±0.1	4.4±0.2
	20 (7.0 Mn)	7.1±0.0	6.5±0.2	5.8±0.2	5.9±0.3	4.8±0.1
15ºC	0 (0 Mn)	1.0±0.1	1.3±0.2	0.8±0.3	0.6±0.2	0.5±0.1
	8 (2.8 Mn)	3.5±0.2	3.8±0.5	3.8±0.9	3.0±0.2	2.7±0.0
	15 (5.2 Mn)	5.4±0.5	4.9±0.1	4.3±0.1	4.4±0.3	3.8±0.2
	20 (7.0 Mn)	7.8±0.2	7.0±0.1	6.7±0.4	6.2±0.2	5.2±0.1
20ºC	0 (0 Mn)	0.1±0.1	0.4±0.3	0.4±0.2	0.4±0.2	0.4±0.0
	8 (2.8 Mn)	2.6±0.1	2.6±0.2	2.4±0.1	2.6±0.4	2.7±0.3
	15 (5.2 Mn)	4.5±0.1	4.3±0.2	4.1±0.2	3.9±0.1	3.9±0.5
	20 (7.0 Mn)	6.4±0.2	6.1±0.2	5.9±0.2	5.5±0.4	4.7±0.1

EarthTec QZ Trials

Veligers were eventually susceptible to EarthTec QZ. The highest mortality achieved in these trials was with the 16.7 uL/L concentration after 24 hours at 15ºC (Table 9). Data from the beaker where the pH probe broke was censored from analysis. Temperature was a weak significant variable in predicting mortality (F(2):3.11; p-value: 0.047). At 10ºC, mortality was not different enough to show that increased concentration caused increased mortality. However, duration was a significant variable showing that 24 h had significantly more mortality than the lower duration time periods (F(3):7.92; p-value: <0.001). In the 15ºC trials, concentration was a significant variable (F(3):7.13; p-value: <0.001) showing that as concentration increased mortality increased with control mortality significantly lower than the treatment mortality. Duration was also a significant variable in the 15ºC trial, which showed mortality at 24 h was significantly different from the other durations (F(3):4.662; p-value: 0.007). For the 20ºC trials, concentration was a significant variable (F(3):4.98; p-value: 0.004); mortality at 16.7 µL/L was higher than the lower concentrations (Table 9). Duration was also a significant variable, because mortality at 24 h was significantly different from the other durations (F(3):5.325; p-value: 0.003).

At all temperatures, the 24 h 16.7 µL/L EarthTec QZ trials resulted in the highest mortality (Table 9, Figure 3). The mortality was not significantly different among the different temperature trials. Mortality increased with exposure duration for the 16.7 µL/L trials (Figure 3). At 24 h, the mortality was significantly predicted by concentration (F(3):47.73; p-value: <0.001) where 10 and 16.7 µL/L EarthTec QZ were significantly different from each other and were significantly different from 3 and 0 µL/L EarthTec QZ, which were not significantly different from each other.

Table 9. Average mortality with standard deviation of EarthTec QZ trials tested by temperature. Treatment mortality that was significantly different from the control mortality are represented by *p<0.05, **p<0.01, ***p<0.001. Data from the beaker that had the pH probe break was censored ([a]).

Temp. (ºC)	Earth Tec QZ (µL/L)	1 h	3 h	6 h	24 h
10	0	1.6±0.7%	0.0±0.0%	6.0±1.9%	4.2±1.2%
	3	2.8±2.0%	5.9±3.4%	2.3±1.1%	5.2±1.8%
	10	5.6±4.7%	9.2±6.2%	8.0±6.3%	27.4±5.2%*
	16.7	0.6±1.0%	6.1±3.9%	5.0±3.5%	29.5±11.6%
15	0	3.6±0.9%	4.2±3.5%	3.2±0.6%	3.4±2.8%
	3	2.8±1.8%	14.7±7.5%	4.4±3.4%	4.5±2.9%
	10	3.8±4.0%	12.8±6.4%	[a]13.1±0.2%***	34.2±5.6%**
	16.7	5.0±2.9%	16.4±3.7%*	21.1±11.5%	38.1±11.0%*
20	0	2.3±1.4%	2.5±1.9%	4.5±0.2%	2.2±1.3%
	3	2.8±0.6%	4.7±2.6%	3.9±1.1%	3.8±2.0%
	10	3.9±2.0%	5.8±1.8%	4.8±2.9%	9.5±2.4%**
	16.7	4.1±2.4%	5.2±1.7%	8.5±3.1%	36.6±2.8%***

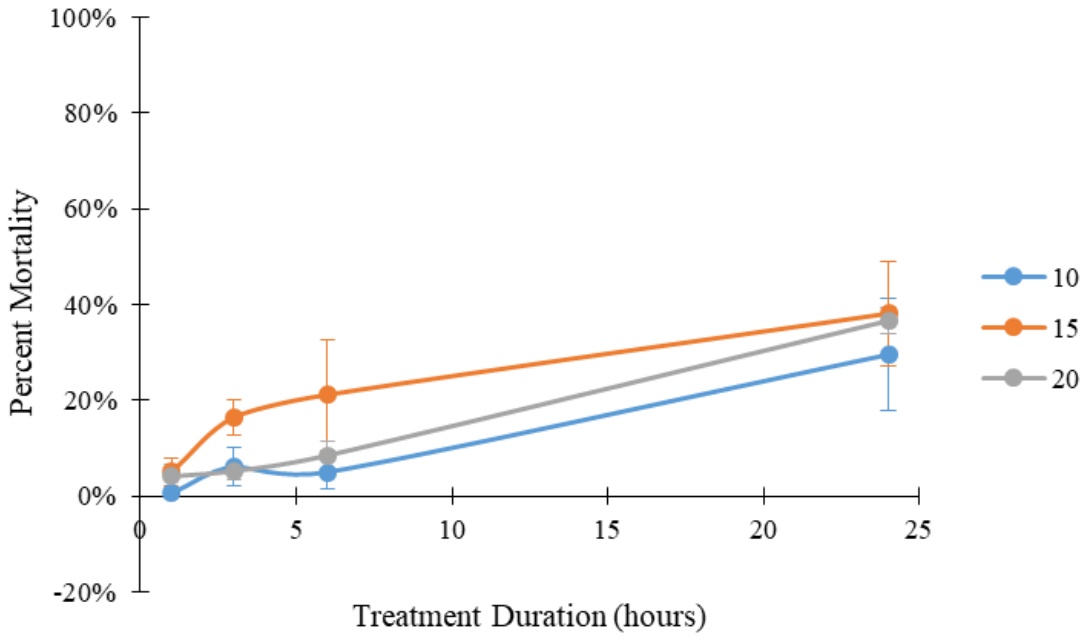

Figure 3. Mortality with standard deviation bars for 16.7 µL/L EarthTec QZ at each tested temperature.

During testing, temperatures stayed consistent, having 1.0ºC variation. At the start of the 10ºC trials, the temperatures were about 14ºC, but obtained 10ºC by 1 h. The 15 and 20ºC tests were at temperature the entire duration. Average dissolved oxygen for all beakers over all testing concentrations and durations was 8.1 mg/L. There was only a slight drop in dissolved oxygen levels to 5.6 mg/L in the control 24 h at 20ºC, but this had no effect on mortality (Table 9). The pH was consistent over time and did not fluctuate; the average pH was 8.30.

Specific conductance did differ between temperatures, indicating that it differed over testing days. During the 10ºC tests, the specific conductance was approximately 1.0 mS/cm at the beginning of testing and increased to 1.1 mS/cm over 24 h (Table 10). For the 15ºC trials, the specific conductance was 0.95 mS/cm and increased to 0.97 mS/cm at 24 h. The 20ºC trials specific conductance was 0.86 mS/cm and increased to 0.92 mS/cm at 24 h (Table 10). Specific conductance did not increase with concentration at any temperature.

Table 10. Average specific conductance (µS/cm) readings for the EarthTec QZ trials tested by temperature.

Temperature	Earth Tec QZ (µL/L)	0 h	1 h	3 h	6 h	24 h
10ºC	0	1009.0	1060.7	1098.5	1111.0	1123.8
	3	997.3	1059.0	1080.6	1123.8	1121.6
	10	1004.0	1091.2	1069.0	1143.5	1114.2
	16.7	1009.0	1102.0	1110.2	1147.1	1122.3
15ºC	0	954.1	967.2	964.4	964.4	969.3
	3	956.1	974.0	973.2	975.8	970.9
	10	956.4	968.7	966.1	976.1	966.6
	16.7	953.2	963.3	964.4	978.3	964.0
20ºC	0	954.6	942.9	931.8	900.3	897.3
	3	953.7	907.0	897.1	870.7	889.0
	10	875.3	1026.8	1031.6	888.6	909.7
	16.7	874.5	921.4	922.3	861.6	877.8

The 10 and 20ºC EarthTec QZ stock solutions were made the morning of treatment and the 15ºC stock solutions were made the night prior to testing. The copper concentrations did not change in the morning. Copper values were measured to determine the concentration of EarthTec QZ. The desired value of copper in the stock solution was in accordance with manufacturers recommendations (Table 11). The amount of product added into the stock solutions represented a 1.1X concentration of copper (Table 11). No adjustments were necessary.

Table 11. Process of adding EarthTec QZ to make a 1.1X EarthTec QZ stock solution, where total and free copper readings were evaluated.

Temp (ºC)	EarthTec QZ (µL/L)	Added (uL)	Free Copper (mg/L)	Total Copper (mg/L)
20	3 (0.2 Cu)	23	0.22,0.22	0.23,0.23
	10 (0.66 Cu)	77	0.67,0.67	0.69,0.71
	16.7(1.0 Cu)	128	1.10,1.10	1.11, 1.13
15	3 (0.2 Cu)	23	0.20, 0.19	0.19, 0.20
	10 (0.66 Cu)	77	0.68, 0.67	0.67, 0.69
	16.7(1.0 Cu)	129	1.09,1.09	1.10, 1.10
10	3 (0.2 Cu)	23	0.21,0.22	0.21, 0.18
	10 (0.66 Cu)	77	0.68,0.66	0.68, 0.66
	16.7(1.0 Cu)	129	1.09,1.09	1.13, 1.10

Total and free copper measurements were very similar, with free copper measurement slightly lower than the total copper readings (Table 12). Copper concentrations did decrease over time in all temperature trials, by approximately 12 to 15% at 24 h. In the 10 and 15ºC trials, the copper concentrations decreased the most within the first hour to 3 hour, and then the concentrations did not decrease as quickly and stabilized (Table 12). In the 20ºC trials, the copper concentration stayed constant or increased for the 1 h, but by 3 h started decreasing. The 24 h copper concentrations were similar at all temperatures (Table 12).

Table 12. Average total and free copper concentrations with standard deviations over time for the trials. The 0 hour was within 10 min of pouring the stock solution in to the veliger containing water in the test beaker.

Temp. (ºC)	Earth Tec QZ (µL/L)	Total Copper (mg/L)					Free Copper (mg/L)				
		0 h	**1 h**	**3 h**	**6 h**	**24 h**	**0 h**	**1 h**	**3 h**	**6 h**	**24 h**
10	0	0.08 ±0.03	0.10 ±0.09	0.09 ±0.02	0.09 ±0.02	0.09 ±0.01	0.11 ±0.03	0.09 ±0.06	0.07 ±0.02	0.09 ±0.02	0.10 ±0.02
	3 (0.2 Cu)	0.23 ±0.00	0.20 ±0.03	0.20 ±0.01	0.23 ±0.02	0.26 ±0.03	0.21 ±0.01	0.22 ±0.02	0.20 ±0.02	0.20 ±0.03	0.27 ±0.03
	10 (0.66 Cu)	0.63 ±0.02	0.59 ±0.01	0.58 ±0.03	0.59 ±0.02	0.55 ±0.02	0.62 ±0.02	0.57 0.03	0.57 ±0.03	0.57 ±0.02	0.55 ±0.05
	16.7 (1.0 Cu)	1.03 ±0.02	0.98 ±0.03	0.93 ±0.02	0.92 ±0.04	0.90 ±0.04	1.02 ±0.01	1.01 0.06	0.93 ±0.03	0.93 ±0.05	0.89 ±0.02
15	0	0.16 ±0.02	0.14 ±0.02	0.07 ±0.01	0.06 ±0.02	0.08 ±0.01	0.15 ±0.00	0.13 ±0.03	0.08 ±0.01	0.08 ±0.02	0.06 ±0.02
	3 (0.2 Cu)	0.30 ±0.01	0.29 ±0.02	0.25 ±0.03	0.23 ±0.02	0.23 ±0.03	0.30 ±0.00	0.28 ±0.01	0.25 ±0.02	0.26 ±0.03	0.22 ±0.03
	10 (0.66 Cu)	0.70 ±0.02	0.66 ±0.01	0.58 ±0.03	0.59 ±0.04	0.60 ±0.02	0.70 ±0.02	0.65 ±0.01	0.57 ±0.02	0.58 ±0.03	0.60 ±0.03
	16.7 (1.0 Cu)	1.10 ±0.02	1.00 ±0.05	0.93 ±0.01	0.97 ±0.02	0.93 ±0.02	1.08 ±0.01	1.00 ±0.04	0.94 ±0.01	0.97 ±0.02	0.91 ±0.03
20	0	0.19 ±0.05	0.23 ±0.02	0.19 ±0.01	0.08 ±0.01	0.09 ±0.04	0.19 ±0.06	0.22 ±0.03	0.19 ±0.02	0.09 ±0.03	0.07 ±0.03
	3 (0.2 Cu)	0.33 ±0.08	0.37 ±0.02	0.33 ±0.02	0.20 ±0.04	0.24 ±0.01	0.33 ±0.08	0.36 ±0.02	0.30 ±0.02	0.20 ±0.05	0.24 ±0.02
	10 (0.66 Cu)	0.72 ±0.11	0.76 ±0.01	0.65 ±0.02	0.58 ±0.02	0.65 ±0.02	0.71 ±0.11	0.76 ±0.02	0.66 ±0.02	0.57 ±0.02	0.62 ±0.02
	16.7 (1.0 Cu)	1.10 ±0.09	1.10 ±0.02	1.01 ±0.03	0.89 ±0.02	0.91 ±0.01	1.09 0.08	1.09 ±0.03	1.02 ±0.03	0.88 ±0.03	0.91 ±0.03

EarthTec QZ with Potassium Chloride

The additional testing with EarthTec QZ and potassium chloride resulted in no significant mortality compared to control (Table 13). These tests were conducted at 20ºC and trials were within ±1ºC. Dissolved oxygen levels were between 7 and 8 mg/L with an average of 7.62 mg/L. The pH did not vary and the average was 8.22. Conductivity increased with additional potassium chloride. The 50 mg/L KCl specific conductance was an increase of 89 µS/cm over background and the 400 mg/L KCl treatment had a specific conductance of 1.603 mS/cm, which was an increase of 728 µS/cm. The copper concentrations after an hour of treatment time were lower by about 0.05 mg/L, which was similar to the no KCl treatments (Table 12).

Table 13. Average mortality with standard deviation of EarthTec QZ trials with additional potassium chloride (KCl).

EarthTec QZ (µL/L)	50 mg/L KCl	400 mg/L KCl
0	3.6±0.6%	3.5±0.8%
3	2.5±1.5%	5.1±1.1%
10	2.4±0.8%	5.7±0.4%
16.7	3.1±1.6%	4.1±0.0%

Analytical Laboratory Water Chemistry

The turbidity and the chemical oxygen demand (COD) results for the testing water was low (Table 13) and did not have much variation. When there was no veliger concentrate in the samples the turbidity was <1 NTU and COD was undetectable. With the addition of veligers turbidity and COD increased (Table 13). The amount of veliger concentrate did not correlate with the measurement of turbidity or COD. Observationally, the algae content in the veliger content was highest on 7/8 and high throughout that week, which was when there were higher COD readings.

COD and turbidity were not correlated to each other as represented with replicates 3, 4, 5, and 8, the turbidity varies as much as the COD (Table 14). High COD measurements were not correlated to the trials with low mortality.

Table 14. Measurements of turbidity and chemical oxygen demand (COD) of water used during the trials. When veliger concentrate was added it was the same amount as was added the testing beakers for that day's trial.

Date	Rep	Trial Conducted	Veligers added (mL)	Turbidity (NTU)	Veligers added (mL)	COD (mg/L)
6/25	1	Chlorine 10ºC	0	0.5	4	30
6/26	2	$KMnO_4$ 10ºC	0	0.4	4	50
6/27	3	$KMnO_4$ 15ºC	4	23.2	4	30
7/8	4	$KMnO_4$ 20ºC	8	4.7	8	40
7/9	5	EarthTec 20ºC	10	10.2	10	60
7/10	6	EarthTec 15ºC	0	0.6	6	50
7/22	7	EarthTec 10ºC	0	0.5	0	<20
7/23	8	Chlorine 20ºC	7	3.3	7	50
7/24	9	EarthTec 20ºC	0	0.9	0	<20

Adult Mussel Testing Different Source Waters

Trial 1

There was a large mortality event with the adult mussels in the first week of testing in both the surface and well water treatments (Figure 4). After the first event, there was continuous mortality in both the surface and well trials. There was no significant difference in the mortality between the two water treatment types; average final mortality for surface was 76% and well was 73% at 48 days. Using the linear trend of these two source waters, the date of 100% mortality for surface water was November 6, 2019 and well water was March 16, 2020. This is based on the assumption that temperature is constant, resources are not limited (i.e. water changes still occur) and water quality is consistent. This prediction shows that it would take up to a year for all of the mussels to die.

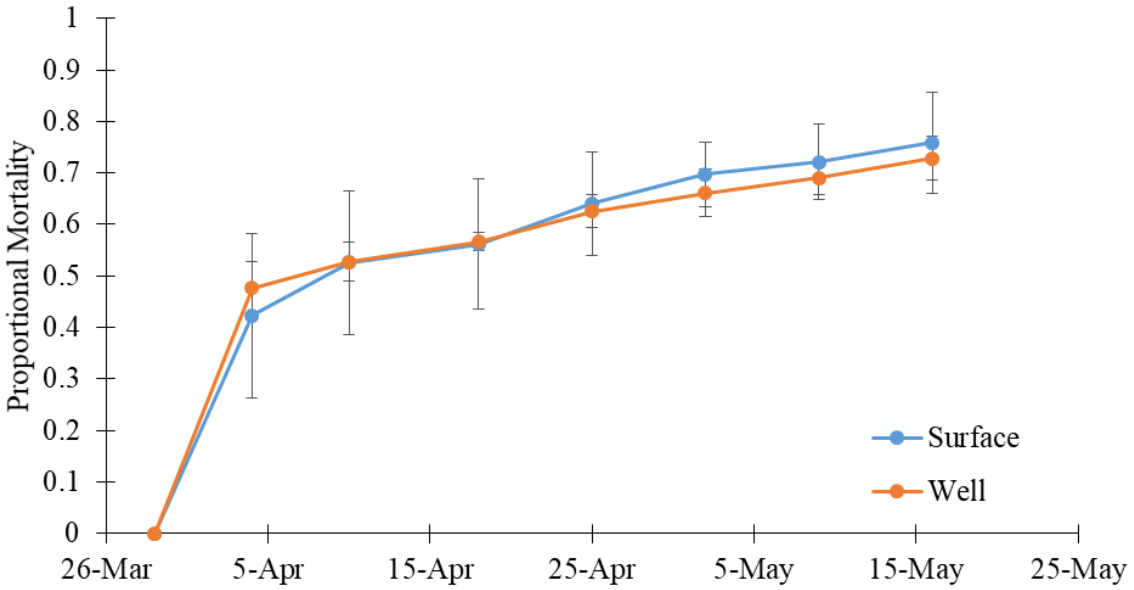

Figure 4. Average proportional mortality of adult mussels in trials starting March 29 in surface and well water.

Water quality measurements with the Hach probe showed a temperature of 26.6 and 27.0 ºC in the surface and well treatments on April 1. Average water temperatures were 24ºC for trial 1. Dissolved oxygen remained high with an average of 8.57 mg/L, which supported the mussel survival. Water changes lowered the pH, but pH increased with time and presence of mussels. The range of pH was 7.22 to 8.92 for surface water and 7.71 to 9.00 in well water. Specific conductance was similar between the two water types. The average surface specific conductance was 1.1 mS/cm and 1.2 mS/cm for well water.

Water temperature was monitored hourly in one of the treatment boxes for both surface and well with a HOBO meter. This data showed that water temperatures were over 30ºC for two days in a row the first week of the trial (Figure 5). After the high temperature reading from the Hach probe, we turned on the air conditioner in the trailer, which kept the test coolers at a more constant temperature. The average testing temperature was 24.5ºC in the surface cooler and 24.4ºC in the well cooler. Drops in the temperature indicated water changes, as the water used was stored inside a colder facility.

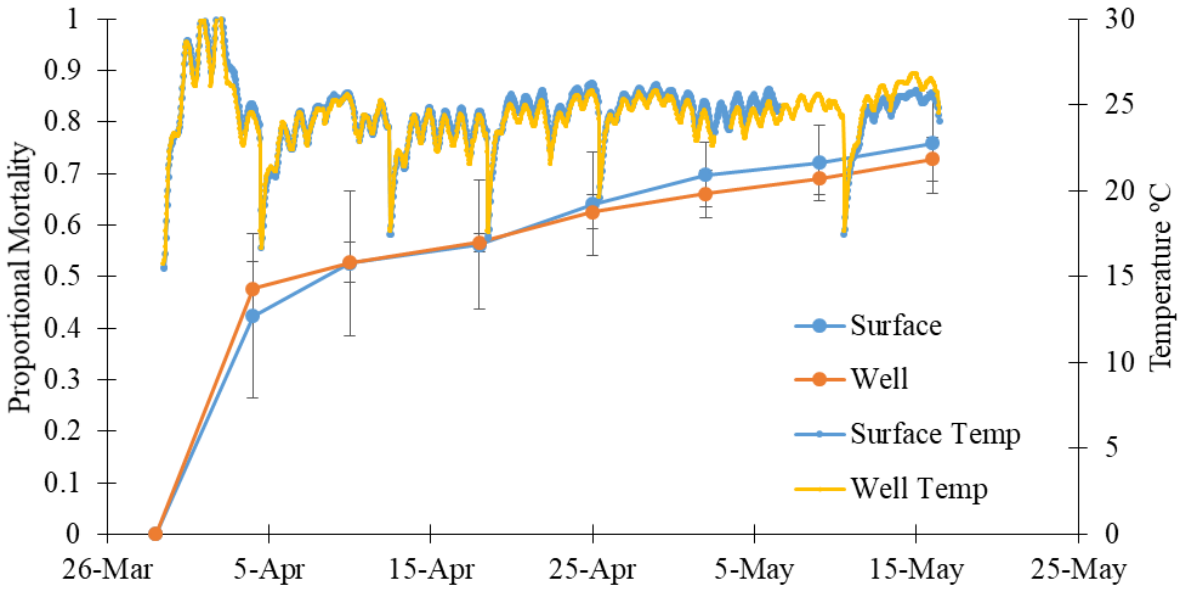

Figure 5. Average proportional mortality with HOBO temp loggers for the surface and well water trials.

The adult mussels that were measured and used in testing were approximately the same size for both treatments, with an average of 19.5 mm (Figure 6a). Mussel size was not a factor in mortality over time (Figure 6b). For example, the small ones did not die faster than the larger mussels in either water source treatment. The average length of mussels living at the end of treatment was very similar to the average tested (Figure 6a).

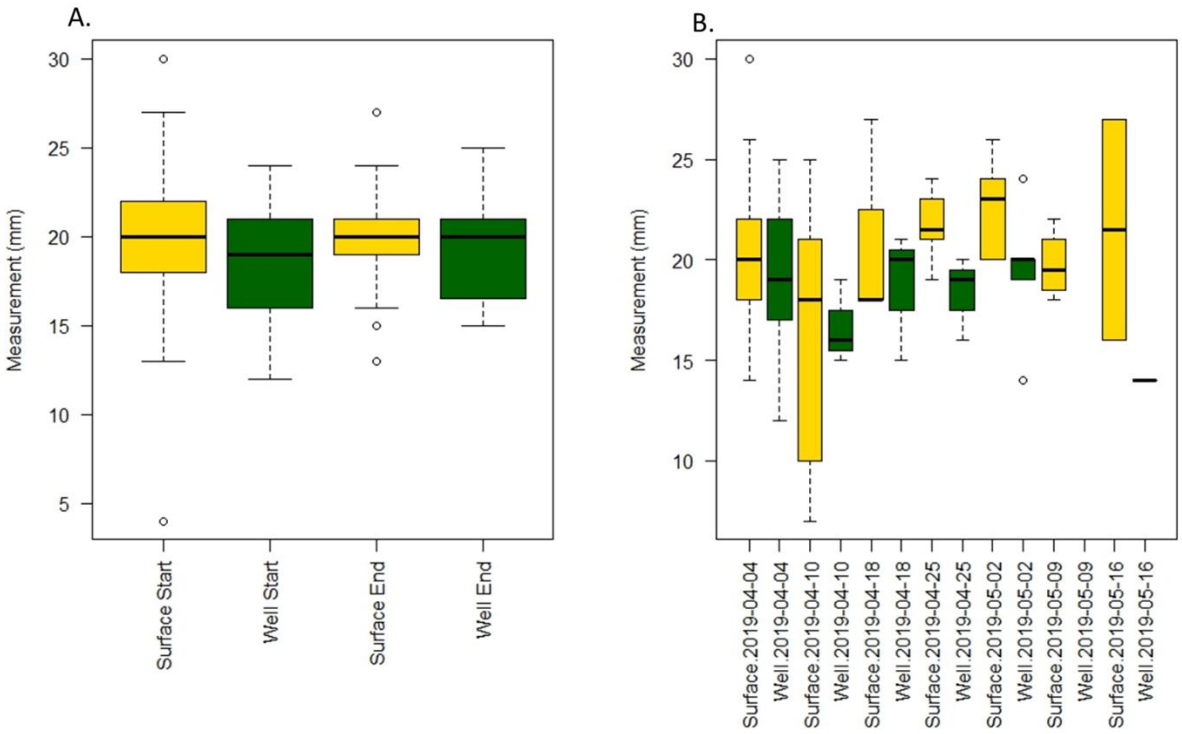

Figure 6. Boxplot of the measurements of A. Live adult mussels at the beginning of the trial and at the end of the trial for each water type and B. Dead adult mussels over the duration of the trial. Gold represents surface treatment and green represents well treatment.

Trial 2

In trial 2, there was also a large mortality event within two weeks of initiating testing (Figure 7). The mortality was similar in all of the water sources. At the 6/28 mortality check, the lake treatment had significantly higher mortality than the surface treatment mortality. By the end of the testing period, check day 7/25, the lake water treatment had significantly higher mortality than the well and surface water mortalities (Figure 7). Final mortality, at day 70, in the lake water treatment was 98%, well water treatment mortality was 89% and surface water treatment mortality was 81%.

Water quality measurements with the Hach probe showed that the average temperature was higher in the lake water trials, where average lake temperature trials were 26.2ºC, surface water trials were 24.3ºC and well water trials were 25.4ºC. Dissolved oxygen was above 7.0 mg/L. The lake source water was very low (<5.0) in dissolved oxygen for water changes, but by the next check day the dissolved oxygen would be above 8.0 mg/L. This could have compounded the stress on the mussels in addition to the high temperatures. The pH did not vary much over time or between water sources. Average pH during treatment was lake 8.66, surface 8.51, and well

8.65. Lake water had the lowest specific conductance and well water had the highest (Figure 8). Specific conductance slowly decreased over time in the lake water. The surface and the lake water decreased slightly over time until July 5, where the surface water increased specific conductance and the well water specific conductance continued to decrease (Figure 8).

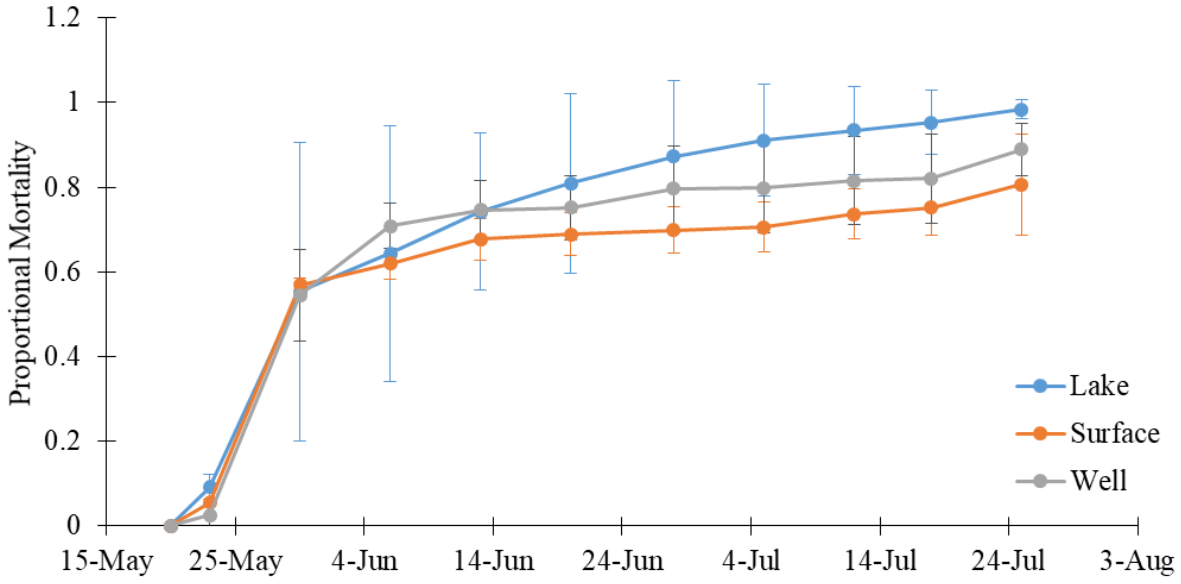

Figure 7. Average proportional mortality of adult mussels in trials starting May 20 in lake, surface, and well water.

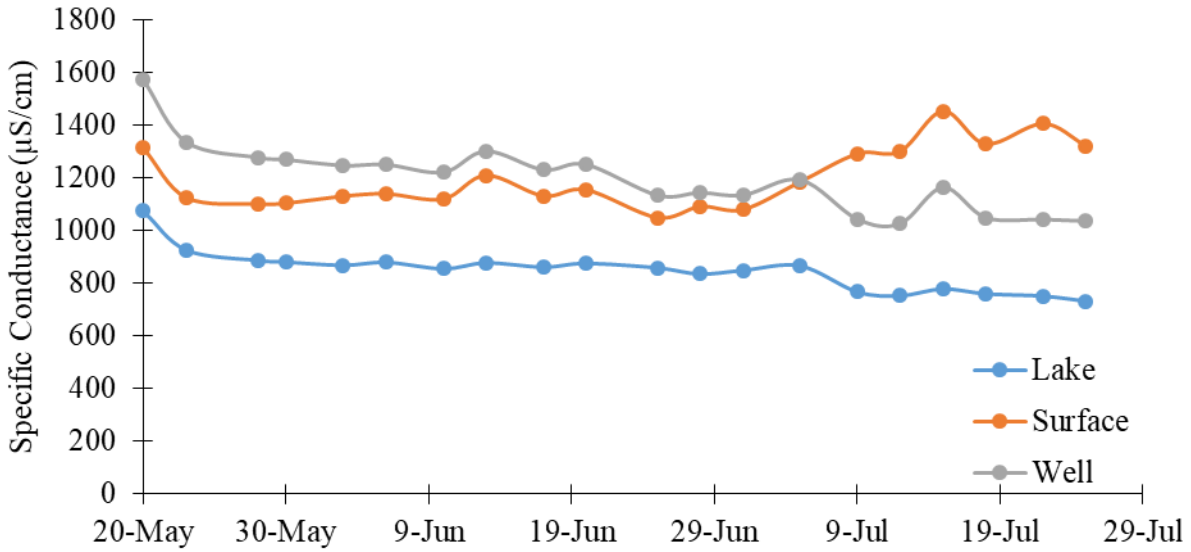

Figure 8. Average specific conductance for test water from each source water over testing period.

Water temperatures were monitored hourly in one of the treatment boxes for lake, surface and well with a HOBO meter. This data showed that water temperatures were over 27.5ºC for 2 days around May 25. When the temperatures were greater than 30ºC (the red line on Figure 9), which was close to the lethal limit for quagga mussels (McMahon 2011), then mortality in that treatment increased substantially. The lake treatment boxes were at the back of the trailer furthest from the air conditioner and had higher temperatures over the testing period.

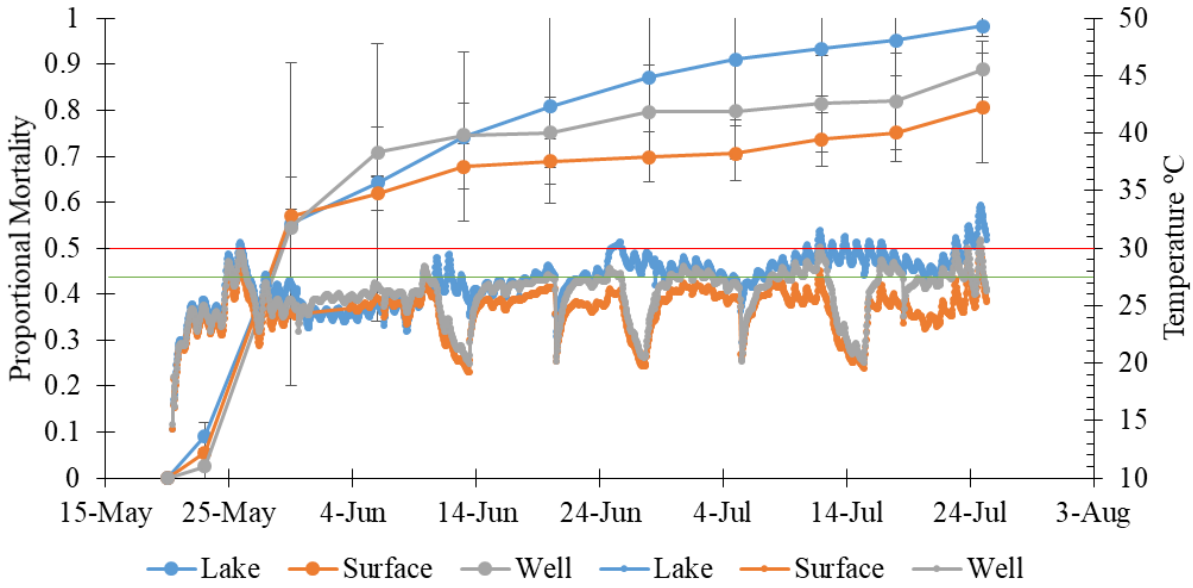

Figure 9. Average proportional mortality with HOBO temp loggers for the lake, surface and well water trials. The red line represents 30ºC and the green line represents 27.5ºC on the graph for reference.

The adult mussels in one cup per replicate testing configuration were measured and used in testing were approximately the same size for all treatments, with an average of 16.6 mm (Figure 10a). Mussel size was not a factor in mortality over time (Figure 10b). For example, the small ones did not die faster than the larger mussels in any of the water source treatments. The average length of mussels living at the end of treatment was very similar to the average tested (Figure 10a), though the sample size is small.

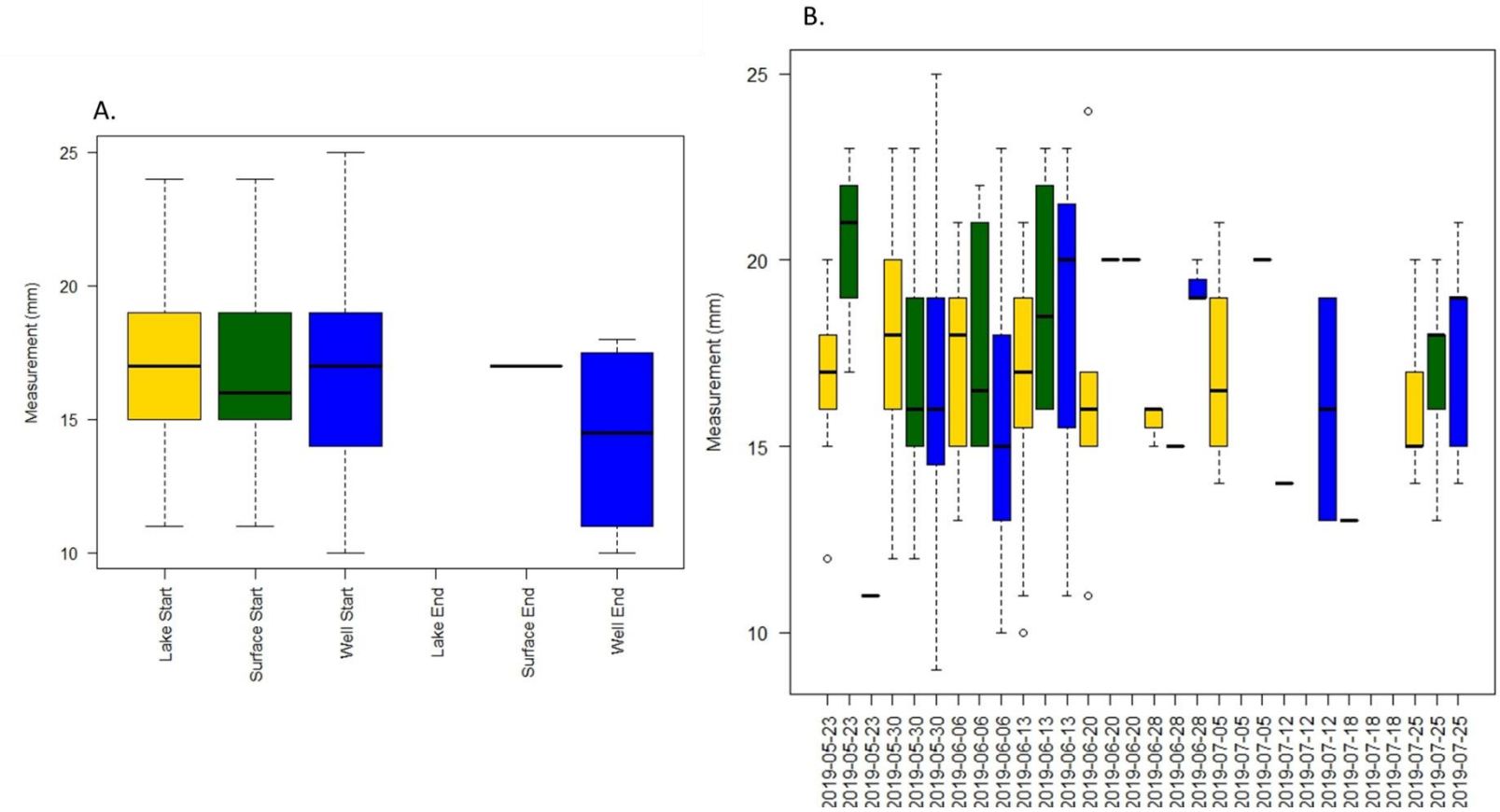

Figure 10. Boxplot of the measurements of A. Live adult mussels at the beginning of the trial and at the end of the trial for each water type and B. Dead adult mussels over the duration of the trial. Gold represents lake treatment, green represents surface treatment and blue represents well treatment.

Discussion

Chlorine

The maximum mortality of quagga mussel veligers achieved with chlorine in 24 h was 52% in the 10 mg/L trials at 10ºC (Table 1). The actual chlorine dosage in this trial was 6.6 mg/L as total chlorine (Table 4). The 20ºC trial had a higher actual chlorine dosage at 9.7 mg/L total chlorine, but had less mortality. In the 10ºC trials, the decay rate of chlorine was slower than in the 15 and 20ºC trials (Table 4). This could have contributed to the higher mortality, though the differences in mortality were not significant among the 24 h exposure durations between temperatures.

The chlorine stock concentrations were the most difficult to make (Table 3). Once added to the veliger concentrate, the amount of chlorine was much less than expected (Table 4). The chlorine disappeared quickly from solution. A colorimeter was used to determine the concentrations of total and free chlorine in the trials. The colorimeter gave expected results, but some results were higher than expected, such as the 24 h readings during the 10ºC trials (Table 4). The standard deviation was not too high for these tests, showing that all three replicates read similarly. Possible sources of error could have been the machine was calibrated to a different measurement or the glassware was dirty and increased the readings. Those readings seem to be the only ones that were possible errors.

In previous studies, chlorine was shown to be effective on adults at 0.5 to 2.0 mg/L with treatment durations of up to 4 weeks and end of pipe readings at 0.5 mg/L free chlorine (LePage 1993; Van Benschoten et al. 1993a; Sprecher and Getsinger 2000). This was dependent on temperature, water chemistry, and physiological state of the mussels. They also indicated that chlorine treatments would work to reduce settlement. This study showed that chlorine was not effective at killing veligers in durations shorter than 24 h and that a high concentration (6.6 mg/L Cl) was needed to obtain significantly higher mortality from control. Klerks et al. (1993) discussed that veliger testing with chlorine has not been widely investigated and mentioned that after a 1 h duration at 7.5 mg/L chlorine veligers were still alive. This study confirmed this and showed that it took 24 h to obtain 50% mortality.

The high concentrations used in this study are not economically feasible and potentially crop damaging. Many plants are chloride sensitive, including many grown by UWCD customers, such as strawberries, citrus, almonds, melons, etc. (Ayers and Westcot 1994; KALI 2017). Chlorine is the ion form and chloride is the electron rich chlorine atom. By introducing chlorine into the irrigation water, there is a higher potential of chloride ions forming in the soil and becoming toxic to the plants. In these trials, the specific conductance increased over time at the 10 and 15ºC trials (Table 2), indicating that the chlorine molecules were breaking down into chlorides. At 20ºC, the specific conductance did not increase with time, indicating that the chlorine was dispersing out into the air.

Large concentrations of chlorine were needed to achieve mortality effect on veliger mussels (Table 1). This study did not look at the veligers during treatment, so it was possible that low doses of chlorine would be effective at preventing settlement. If the veligers do detect the chemical and close up, they would not settle on the infrastructure being treated. However, once

the chlorine concentration dissipates, or is diluted, the veligers are free to settle and establish new colonies. This is occurring in California waterways, as Metropolitan Water District treats their water with chlorine that is delivered to Orange County Water District (OCWD). OCWD has found multiple live adult mussels in their basin.

Potassium Permanganate

Veliger mortality was 41% after a 24 h exposure to 20 mg/L potassium permanganate at 20ºC (Table 5, Figure 2). There were differences in mortality with temperature, but age of stock concentration was a better explanatory variable. The stock solution for trials at 10 and 20ºC were made the night before testing and resulted in higher mortality than the 15ºC trials that the stock solutions were made the morning of testing (Figure 2, Table 7). Final manganese readings were similar for all trials (Table 7). In the 20ºC trials, 15 and 20 mg/L had similar mortalities over all exposure durations. These concentrations were dosed correctly as the spectrophotometer readings were as expected (Table 8).

Water chemistry readings were within tolerance limits of the survival of veligers. The specific conductance did not increase with duration or concentration. This indicated that there were not enough salt ions added to change the conductivity of the solution. Therefore, the potassium ion was not contributing to the toxicity of the permanganate at these concentrations. Potassium permanganates mode of action is more likely due to the oxidative effect than the possible effects of potassium toxicity. Potassium toxicity in mussels includes changing the electrolyte balance within cells disrupting homeostasis (Moffitt et al. 2016).

Manganese readings were conducted with the spectrophotometer to ensure that the concentrations of chemical were correct. Decay of manganese was assessed, but the manganese did decrease with time, but the decay of active potassium permanganate was not assessed. In the future, it is recommended that the amount of potassium permanganate be recorded, which would use standard spectrophotometric method for potassium permanganate of reading the vials at a wavelength of 525 nm (APHA 2012). Observational evidence shows that the concentration of potassium permanganate decreased over time, as the color in the 24 h was not pink but brown.

Previous studies with potassium permanganate showed variable results on zebra mussel veligers. At 2.5 mg/L with 1 h exposure durations, the veliger mortality was 36%, but was not consistent across longer treatment times (AWWA 1997; Sprecher and Getsinger 2000). This study showed that around 30% mortality was achievable independent of duration time at 20ºC at high concentrations (Table 5). Another study showed that 8 mg/L of potassium permanganate with a 3 h exposure duration resulted in 60% mortality of zebra mussel veligers (Coyle et al. 2014). This study did not achieve 60% mortality even at 20 mg/L potassium permanganate. Klerks et al. (1993) found that static treatments at concentrations of 2.5 mg/L or less resulted in <30% mortality for up to a 3 h duration period. They did show that settlement was greatly reduced in treated bioboxes, hypothesizing that the veligers detect the chemical, close up, and pass through. Klerks et al. (1993) results were similar to this study, but had success at lower concentrations. Mussels that had cracks or could not close all of the way during the chemical exposures were more likely to be dead.

Potassium is a key nutrient for plant growth and potassium permanganate has low toxicity to most plants. However, concentrations greater than 2 mg/L is harmful to fish and other aquatic organisms (Hobbs et al. 2006). This study tested very high concentrations relative to that tested in the published literature 20 mg/L compared to 2 mg/L, but did not achieve 100% mortality. Treatment using potassium permanganate at higher doses may not be effective, cost prohibitive and environmentally toxic.

Copper

The highest mortality achieved with EarthTec QZ was 38.1% in the 16.7 uL/L concentration after 24 hours at 15ºC (Table 9). Mortality was similar and not significantly different across all temperatures in this concentration at 24 h. Higher concentrations increased the mortality of veligers; however, there was not much difference in mortality between the 10 and 16.7 µL/L concentrations of EarthTec QZ (Table 9). Copper concentrations were as expected at the start of the trials and decreased slightly over the trial period (Table 12).

EarthTec QZ stock solutions were the easiest to make and have the copper concentrations read as expected (Table 11). No additional chemical was added after initial stock concentrations were made. The copper concentrations held consistent overnight prior to use. Water quality was as expected for all of the trials and had no influence on the trials.

Addition of potassium chloride into the stock solutions at 50 and 400 mg/L was not effective in increasing the mortality of EarthTec QZ with a 1 h exposure duration. The spill of KCl from the Hach probe resulted in a specific conductance reading of 1.2 mS/cm in the one beaker, which was an increase of 0.21 mS/cm. For reference, the 50 mg/L addition of KCl increased the specific conductance by 0.1 mS/cm and the 400 mg/L addition increased the specific conductance by 0.7 mS/cm. After discussion with Hach about their probes, the KCl concentration that was in the spilled beaker was 3-4 mL of 4M KCl solution. Our trials tried to duplicate the addition of the KCl to achieve 40% mortality with a short exposure duration. Just adding KCl to the stock solution, did not increase the mortality. This investigation was kept short to ensure that all testing was completed and within budget. Another option to consider in the future could be a late addition of KCl after copper treatment, especially if copper is already affecting the veligers. Alternatively, other chemicals in the probe could be made more toxic when combined with EarthTec QZ. The Hach probe also contained silver chloride and the rod inside the probe was platinum, these in combination with EarthTec QZ may be more toxic.

Watters et al. (2013) showed that EarthTec QZ is effective on veligers at 3 ppm in 30 min. This study could not verify those results, but showed that veliger mortality occurs over time. Copper effects the ciliary activity of gill tissue in mussel (Brown and Newell 1972; Jorge et al. 2013). Presence of copper continually degrades ciliary activity and causes imbalances in the sodium levels and inhibition of $Na^{+}K^{+}$-ATPase activity (Jorge et al. 2013). Mortality was thought to occur at a point where the sodium levels could not be rebalanced (Jorge et al. 2013). Given enough time, mortality will occur with continuous exposure to copper.

Interestingly, potassium ions also effect the gill tissues slowing the ciliary activity in zebra mussels (Fisher et al. 1991). Moffitt et al. (2016) confirmed that the exposure to potassium

caused an electrolyte imbalance, which resulted in death. Perhaps what happened in the beaker with the broken pH probe was the veligers were weakened with the copper after a 6 h duration and the elevated potassium intensified the reduction of sodium ions causing elevated mortality to occur.

Many plants and aquatic animals are sensitive to copper at concentrations of 0.1 to 1.0 mg/L copper (Bureau of Reclamation 2005; Jorge et al. 2013). With selective use of EarthTec QZ mortality of fish and native mollusks can be limited (Hammond and Ferris 2019). Drinking water contamination must be below 1.0 mg/L in the state of California (State of California 2018). EarthTec QZ is stable over time and is toxic to quagga mussels. Low doses of EarthTec QZ can be used effectively with a long enough exposure duration, but continuous treatment in UWCD is not feasible due to the toxicity to crops that copper poses.

Veliger Toxicity Trials

The highest mortality observed was with the chlorine trials at 10ºC (Figure 11). However, the highest mortality observed for each chemical was not significantly different from each other at 24 h. This study showed that with any of the three chemicals tested about 50% mortality would be achieved in 24 h.

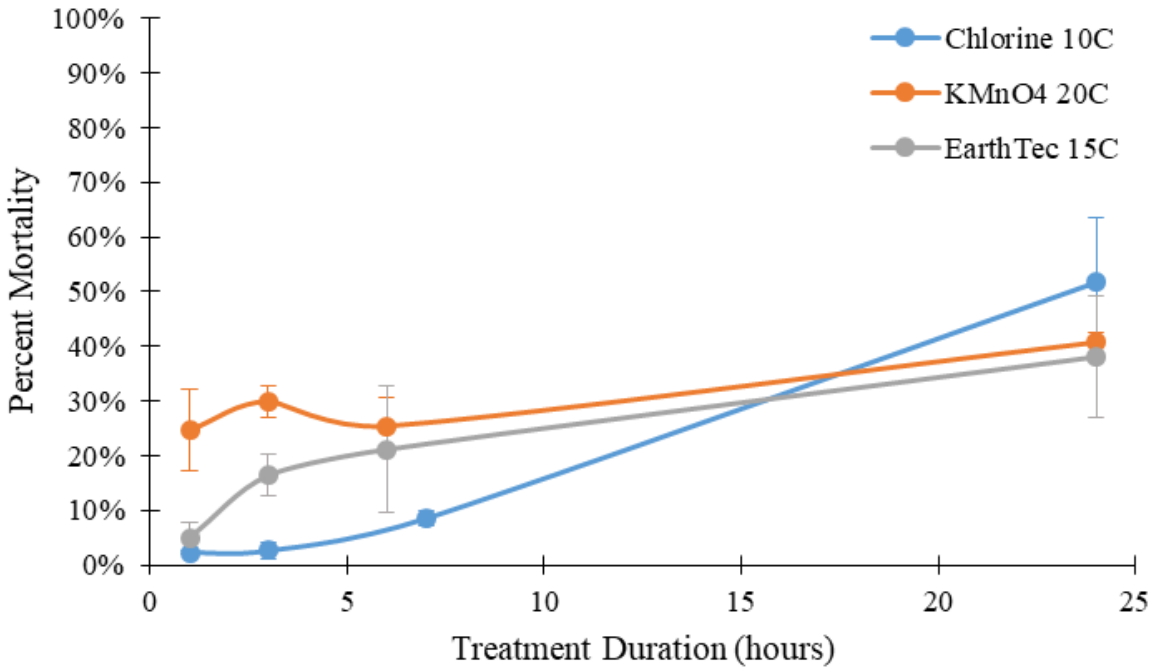

Figure 11. Of the three chemicals tested, the trial with the highest mortality at 24 h is plotted to compare the success of the chemical.

Chlorine and potassium permanganate are oxidants, which the veligers sense and close up to avoid the toxicant. These two chemicals also degrade quickly in the environment and more chemical is needed to maintain the concentrations over time. Copper is not detected by the veligers, it just takes time to penetrate the mussel's cells and cause enough damage to cause death. EarthTec QZ has the most potential to have deleterious effect on veligers with additional contact time without additional chemical being added, which works in static treatments or low flow situations.

Control mortality was low in all trials at all temperatures for all exposure durations. The highest control mortality was 6.6% in the chlorine trial at 15ºC at 3 h. This was background mortality of the veligers because water quality characteristics during testing were within acceptable ranges for maintaining veliger life. Specific conductance varied over testing day, by a range of 0.3 mS/cm and generally decreased over time.

The analytical laboratory water chemistry showed that the water used for testing had very low turbidity and very low chemical oxygen demand (COD). The differences in the turbidity and COD were not significant over time (Table 14). Mortality was not predicted by the slight variations in turbidity or COD. If these chemicals are to be used in higher turbid and COD water, the expected mortality will decrease and additional chemical will be needed to be used for treatment. Further testing and monitoring would need to occur to determine the linear relationship for these parameters.

It is known that veligers close up when they detect an oxidant and do not settle on the infrastructure being treated. This study did not assess the effect of chemical on the veligers during treatment, but this study used a dye to clearly distinguish dead from live veligers ensuring that the data was accurate and robust. Other studies with these chemicals have relied on observation of the veligers, which is cumbersome and inaccurate, resulting in studies evaluating a small number of veligers or error in assessing mortality. This could be the reason for the discrepancies of our study from previous studies. Water quality conditions could also be an important variable influencing the mortality of the quagga mussel veligers. Most of the documented research on chemical control of veligers is with zebra mussels in much different water quality parameters compared to this study.

Adult Mussel Testing in Different Source Waters

The three different source waters did not yield different mortality results with adult mussel survival. There was no difference in mortality of adult mussels between trial 1 and trial 2. Mussel mortality was highly correlated with high temperatures in the testing systems. Multiple events occurred where the temperature was greater than 27.5ºC (Figure 5 and 9), which is the upper thermal limit of quagga mussels (McMahon 2011). However, some mussels survived when temperatures were greater than 30ºC or when temperatures were sustained above 27.5ºC.

This system design kept the dissolved oxygen high and ammonia levels low to simulate flow through water conditions, but could not keep the temperature stabilized. The increasing

temperature over time shows how temperature increases mortality. If temperatures increased to similar levels within the supply pipe; it would be expected to see similar results given the water source. Temperature of the supply pipe will be affected by soil temperature at the depth buried and the supply water temperature.

Specific conductance was different for the three water sources. Lake water had the lowest specific conductance and well water had the highest. Toward the end of the second study, the surface water specific conductance increased to levels higher than the well water (Figure 8). This could be explained by labeling error or by different conductivities in the systems with different barrels of water. The water from the surface and the well water were pumped at the beginning and middle of the study and put into 55 gal barrel drums. One of the drums could have been mislabeled, but equally as likely is that the second pumping of water was slightly different in conductivity from the first pumping. At around this time, the mortality in the surface water increased slightly. The increased conductivity in the surface water could have further challenged the adult mussels already stressed by the warm temperatures (Figure 7 & 8). For the surface water tests, the temperature was lower than the well and lake water during this period.

Prospective Testing Recommendations

UCWD has been charged with ensuring that no mussels reach their customers. AECOM conducted a feasibility study of the Freeman Diversion Facilities and possible control tools. This study investigated the efficacy of the chemical control tools. The AECOM (2016) report also discussed non-chemical tools. They provided locations for the control tools in the UCWD infrastructure. The following is KASF Consulting's recommendations for not delivering live veligers to customers influenced by the results of this study.

Chemical Control Tools

The effectiveness of chlorine is dependent on temperature, contact time, concentration and pH (MSU 2001). Chlorine is most effective as a toxicant in neutral pH (6.5 to 7.5) and loses effectiveness with higher pH; at lower pH the corrosiveness increases and effectiveness is decreased (MSU 2001). The pH of Lake Piru water used in chlorine testing was >8.4. In the trials, where chlorine was a successful treatment, the pH is closer to neutral. For example, Haque et al. (2014) found chlorine was effective on blue mussel (*Mytilus edulis)* larvae at 0.5 mg/L in 120 minutes at 20ºC where pH was 7.8. However, Van Benschoten et al. (1993b) found that 100% mortality was achieved of zebra mussel veligers at 0.5 to 1.0 mg/L for 18 h at 20ºC at a pH of 8.3, which is most cited (Sprecher and Getsinger 2000; Mackie and Claudi 2010). The pH of the Vern Freeman Diversion water in the adult study was 7.6 and well water was 7.9. The pH did increase over time in the barrels. Historically the average pH measurement at Freeman Diversion was 8.4, with a range of 7.4 to 8.4 (AECOM 2016). It would be worthwhile to consider if pH adjustments could be made to the delivered water to increase the effectiveness of chlorine. Meanwhile, it could be advantageous to determine the relationship of chlorine toxicity to quagga mussel veligers at different pH values at a range of temperatures.

Another prospective treatment option is testing how the polymer that helps with desilting applied below the Freeman Diversion effects veligers. Alum or aluminum sulfate as a flocculant at 10-30 mg/L has resulted in removal of the veligers from the water column (Mackie and Claudi 2010). Coupling this treatment with a chlorination treatment improved the likelihood of the veligers becoming part of the floc. Mortality of the veligers in the flocculant did not occur until after 24 h due to suffocation and pH intolerance. Testing this combination would have to be conducted in a flow through system.

Chlorine preforms better than potassium permanganate (Van Benschoten et al. 1993a). Other oxidants have not been proven to work better on veligers, so copious additional research into new oxidants would need to occur before implementing into UWCD infrastructure and veliger control plans. Potassium chloride is not going to be a good option either because of the high conductivity of the water.

EarthTec QZ may be a suitable solution if contact times after UWCD infrastructure are greater than 48 h and can tolerate increased copper loads. However, since the water being delivered is to agriculture users, continuous low levels of copper onto the fields could be detrimental. Instead, EarthTec QZ could be a good tool for UWCD to eliminate an established population.

Other chemicals were discussed in the AECOM (2016) report and determined not to be suitable for use. I agree with this, as the industry accepted chemicals for treating mussels were discussed. Zequanox was not discussed in the AECOM (2016) report, but it has not yet been shown to be effective on veliger life stages. Typical use patterns for Zequanox include periodic treatments and not constant flow though treatments due to the cost of the product.

Assuming that the additional testing is conducted and an effective chemical and dose is deemed appropriate, implementing the chemical control tool should occur. According to AECOM (2016) report use of a chemical feed system would be implemented prior to the desilting basin (Alternative 2a) or prior to Pond B (Alternative 2b). I would suggest implementing the chemical feed at a point below the Moss Screen on the Pleasant Valley Line (Alternative 6). The El Rio facility is protected from veligers if all water from the Main supply line is spread onto the El Rio Recharge Basin. As a note, this does not protect UWCD infrastructure above the chemical feed system. However, UWCD could implement other control tools for its infrastructure in the event of infestation by quagga mussels. Continuous monitoring of veliger mortality after system is implemented would be critical in determining the effectiveness of the control tool.

Physical Control Tools

AECOM's report has an alternative to install a pond infiltration gallery at Pond B (Alternative 3). This seems to be a feasible alternative to chemical treatment. Mackie and Claudi (2010) discuss the use of infiltration galleries and report successful elimination of veligers. As AECOM point out maintenance will need to occur and low turbidity water will increase the longevity of the system and decrease maintenance. RNT Consulting mentioned to KASF Consulting that they have an infiltration gallery that we can use for testing or KASF Consulting is happy to work with AECOM or other engineering firm to build a model to test. This type of testing would be in a flow through system.

There are possible filters that could be installed to filter 75cfs (49MGD). Mud Creek Irrigation District in Michigan looked into infiltration galleries for zebra mussel control, but decided to use filtration methods designed by Amiad Filtration Systems (Power Engineering 1995). KASF Consulting would be willing to work with this system to determine the effectiveness if UWCD is interested. This would also need to be tested in a flow through system.

Conclusions

The chemicals tested in this study did not result in 100% mortality to reach the objective of not delivering mussel veligers to UWCD customers. This study did not achieve the high mortality with low concentrations that other studies achieved. Toxicity of chlorine in this study was impacted by high pH of the Lake Piru water from the dam. High conductivity of Lake Piru resulted in low toxicity of potassium permanganate. Replication of Watters et al. (2013) results with EarthTec QZ has proven challenging (David Hammond, personal communication October 24, 2017). This study showed that use of these chemicals alone is not going to work in the UWCD infrastructure. It is recommended that alternatives be investigated that can be combined with chlorine, such as pH adjustment or use of flocculant with chlorine.

If adult mussels become established in the pipeline, the use of well water or surface water will not kill the mussels. Adult zebra mussels can survive 166 days with no food at 25ºC and up to 945 days at 5ºC (Garton et al. 2014). No published data exists for quagga mussels, but Garton et al. (2014) suggests that they could be more resilient. Veligers do need food to survive and settle. Starving veligers continue to swim and experience mortality within 11 to 15 days at temperatures ranging 12 to 24ºC (McMahon 1996). Therefore, a mix of veliger containing water and well water will not harm the veligers and UWCD customers would have veligers in their systems. In the AECOM report, the use of pumped water from the recharge basin (Alternative 4) would eliminate the risk of veligers in the pipeline.

Further investigation into the proposed chemical treatments or mechanical treatments, such as an infiltration gallery or filters, is highly recommended to ensure that UWCD has feasible options to employ to account for their customers' demands of not delivering veligers. Monitoring at the Moss screen for veligers on a regular basis should be conducted to inform UWCD of potential incidences of veligers passing through some of the natural barriers already in place in the system. These natural barriers include, survival out of the dam into Piru creek, traveling through a gravel bed before and after joining the Santa Clara River at low flows, settling out in the desilting basin or pond b, and filtration at the Moss Screen. Each of these barriers lowers the likelihood of veligers surviving and venturing to customers at the end of the pipelines (Churchill and Quigley 2018). Monitoring these locations for veliger presence could be useful in determining if these barriers are eliminating veligers from UWCD's system.

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Appendix A. Water Quality data for Freeman Diversion and Lake Piru Water in 2019.

This table was generated from data given to KASF Consulting from UWCD's water quality database.

	Water Quality Parameter										
	Calcium (mg/L)	**Magnesium (mg/L)**	**Potassium (mg/L)**	**Sodium (mg/L)**	**Total Hardness as CaCO3 (mg/L)**	**Total Alkalinity as CaCO3 (mg/L)**	**Total Cations (meql)**	**Total Anions (meql)**	**Field pH**	**Copper (ug/L)**	**Manganese (ug/L)**
Freeman Diversion	99.7	36	6	59.7	397	163	10.7	11.2	8.4	13.3*	200*
Lake Piru	73.4	29	4.2	62.3	302	151	8.8	9.1	8.3	-10	23

* indicates an unusually high reading for one sample in the year.