APPENDIX K

Molluscicide Treatment Study

Introduction

Quagga mussels (*Dreissena rostriformis bugensis)* were discovered in Lake Piru, Ventura County, California on December 18, 2013. A Draft Quagga Mussel Monitoring and Control Plan (Plan) has been prepared by United Water Conservation District (United) to comply with State of California requirements under California Fish and Game Code (FGC) §2301. Under FGC, the Plan must define "Methods for control or eradication of adult quagga mussels…" United is interested in pursuing treatment with a molluscicide to achieve extreme population control aimed at quagga mussel eradication from Lake Piru and Piru Creek. However, selecting an effective molluscicide treatment option while also minimizing adverse effects to endangered Southern California steelhead (*Oncorhynchus mykiss*) has proven challenging. Therefore, United initiated a pilot study to test molluscicide options for quagga mussel control in Lake Piru and Piru Creek.

United staff in the Environmental Planning and Conservation Department tested four potential molluscicide options: copper sulfate pentahydrate (EarthTecQZ formulation)(copper), potassium chloride (potassium), citric acid (ZM-X formulation), and carbon dioxide. Copper sulfate pentahydrate and potassium chloride are the only two molluscicides of the four that would be practical for a lake-wide treatment in a lake the size of Lake Piru, therefore the results of these two molluscicides are presented and compared below and the citric acid and carbon dioxide results will be analyzed at a later date for scientific publication.

Methods

General study design

Over the course of four rounds of experiments, quagga mussels were collected; placed in bioboxes (plastic coolers); and tested at low (10°C), medium (17°C), or high (22°C) temperatures with low, medium, or high concentrations of copper or potassium. For potassium, low, medium, and high concentrations were 150 mg/L, 200 mg/L, and 250 mg/L respectively. For copper, low, medium, and high starting concentrations were 60 µg/L, 120 µg/L, and 180 µg/L dissolved elemental copper (dcu) respectively. Because dcu concentrations decrease as copper ions are removed from the water and taken up into biological tissues, dcu concentrations decreased with time from the starting concentration until the copper was refreshed.

Quagga mussels were collected from lower Piru Creek for all experiments and half of the mussels used in the 17°C and 22°C experiments were collected from Lake Piru (see Appendix A for more details on individual rounds of experiments). Mussels were held in five gallon buckets with an aerator and fresh lake water. Mussels were allowed to acclimate to the test temperature for at least 48 hours prior to each experiment and only live mussels were selected for experimental trials after the acclimatization period. One hundred mussels of various sizes over 5 mm were selected, counted, and weighed. Excess water was removed from the outside of the

shells before weighing. One exception of the use of 100 animals per biobox was that only 50 mussels were used for the controls in Round 1a.

Experiments were separated into two different approaches. For the controls and the copper experiment, all groups of 50 or 100 animals per cooler were placed in a single bag submerged in raw lake water (controls) or submerged in the appropriate copper solution in raw lake water with the target copper concentration. Mussels are known to exhibit temporary paralysis when exposed to a sublethal concentration of potassium chloride, therefore a recovery step was implemented for the potassium experiments. For each biobox in the potassium experiment, one hundred mussels were separated into ten bags of ten mussels (100 mussel total) and all ten bags were submerged in the solution. A random bag was selected at regular intervals of time depending on the treatment and the temperature. The random bag was rinsed in raw lake water at the appropriate temperature and then submerged in a "recovery" bucket with fresh, raw lake water at the appropriate temperature with an aerator stone. Animals in the recovery bucket were allowed to recover for at least 48 hours and no longer than 80 hours. The animals in the recovery bucket were then scored as alive or dead following the recovery period.

Dead mussels for all experiments were defined using one of the following four criteria:

1. The mussel shell was gaping open and remained gaping after gently prodding or attempting to close the shell;
2. The mussel shell was closed, but easily popped opened or fell apart with gentle prodding;
3. Any shell that was more than 50% intact and was missing the soft tissue of the animal; or,
4. The two sides of the mussel's shell were completely separated or the shell was crushed without explanation.

If a researcher broke or crushed a shell during handling, then that animal was removed from the analysis and was not scored. This occurred rarely.

Copper

For each round of testing at the different experimental temperatures, twelve bioboxes were divided into three sets of four. Each set of four bioboxes received one of three treatments: 60 ppb, 120 ppb, and 180 ppb elemental copper in the EarthTecQZ formulation in raw lake water. Copper treatments were refreshed with new water and copper every three-five days (See Appendix A for refresh days). Mussels were checked for mortality every three days at 10°C and every day at 17°C and 22°C. During these checks, copper concentrations were measured in a randomly selected biobox from each set of four bioboxes for each copper concentration tested (three bioboxes total per check). Because the bioboxes were filled from the same batch of copper solution for each concentration and because the biomass of mussels was similar, the other three bioboxes in each set of four were assumed to be similar in copper concentrations and were not tested. Copper concentrations were measured during initial setup and during routine checks using

the Copper Porphyrin protocol for the HACH DR 900 Multiparameter Handheld Colorimeter. The sample cells used for the protocol were soaked in a 10% nitric acid bath as needed to remove any buildup on the inside of the glass. Background copper concentrations in the raw lake water in Lake Piru was measured to be 3.6 ppb.

Potassium Chloride

For each round of testing at the different experimental temperatures, twelve bioboxes were divided into three sets of four. Each set of four bioboxes received one of three treatments: 150 ppm, 200 ppm, and 250 ppm lab grade potassium chloride in raw lake water. In Round 1a both potassium and controls were not refreshed during the course of the experiment. In Round 2, potassium treatments were not refreshed while controls were refreshed in conjunction with copper refreshes. After Round 2, the researchers were concerned that refreshing the controls and not the potassium bioboxes would lead to an inflated mortality in the potassium treatment compared to controls and copper treatments, therefore the potassium bioboxes were also refreshed in Round 3 in conjunction with control refreshes (See Appendix A for refresh days). While there may be some variance introduced to the potassium results due to the inconsistency of the potassium refresh protocol, we do not feel that this variance overshadows the main patterns observed in the data and the final conclusions (see Discussion).

Results

Because we are ultimately interested in eradication, we were interested in the end point of 100% mortality across the different treatments. The controls showed little mortality over the course of the four rounds of experiments with less than 10% total mortality for each round. Copper and potassium chloride treatments showed increased mortality rates as temperature increased and as concentration increased (Figures 1 and 2). Most of the experiments were carried out over a duration of 27 days, except copper in Round 3, because the we wanted to test how long the low copper treatment would take to observe 100% mortality at high temperature. Therefore, this test was carried out for 40 days. In the other experiments, copper resulted in 100% mortality most of the time within the 27 day window at medium and high concentrations, however the medium concentration took slightly longer than the high concentration. For potassium, only the highest temperature resulted in 100% mortality within the time tested for medium and high concentrations of potassium chloride. However, the medium temperature/medium concentration, medium temperature/high concentration, and high temperature/low concentration treatments resulted in over 90% mortality in the time tested and if the duration of the experiment had been extended for these treatments, it is possible that 100% mortality would have been observed.

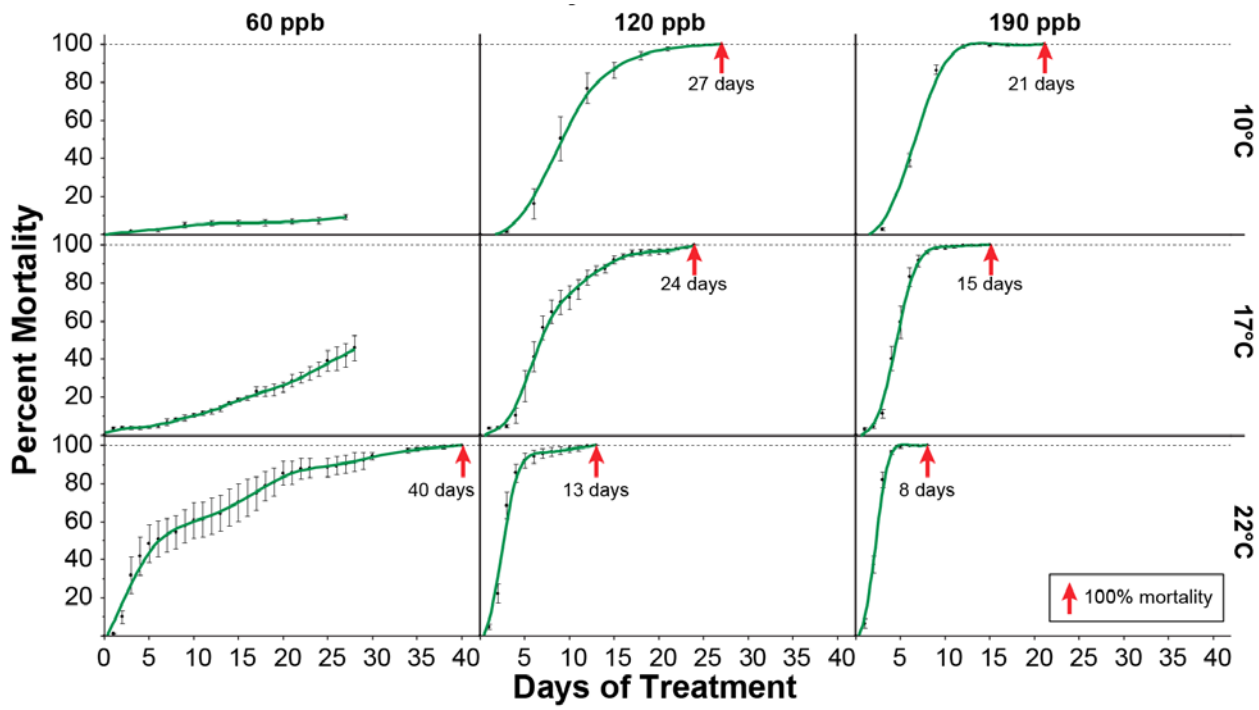

Figure 1. Mortality Curves for Quagga Mussels Treated with Low, Medium, and High Concentrations of Copper Sulfate Pentahydrate EarthTeqQZ Formulation at Low, Medium, and High Temperature

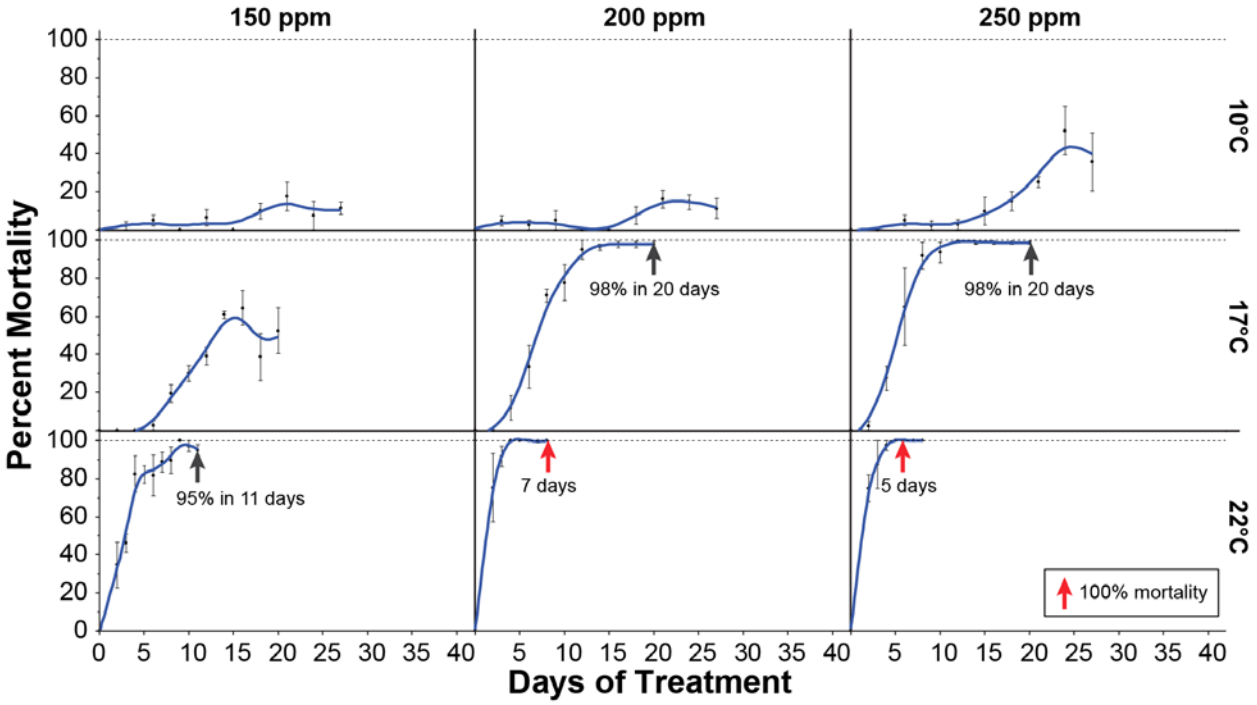

Figure 2. Mortality Curves for Quagga Mussels Treated with Low, Medium, and High Concentrations of Potassium Chloride at Low, Medium, and High Temperature

Discussion

For the durations tested, the copper treatment was effective at achieving 100% mortality across more treatments and tended to be more consistent (i.e., lower variance). The potassium treatments were not as effective at achieving 100% mortality across concentrations and temperatures for the duration tested and the results displayed greater variance in the data. It should be noted that the durations of the potassium chloride experiments were constrained by the number of bags and the time between checks so this resulted in a duration of 27 days at low temperature and 10 days at medium and high temperature. Because potassium chloride does not irreversibly get taken up by biological tissues, like the copper, it is likely that 100% mortality would have been observed at least in some of the treatments if the duration of the experiment had been longer. Either way, the results suggest that copper treatment will be a more cost-effective approach to treating quagga mussels, because the copper works at much lower concentrations than the potassium chloride.

The temperatures we originally targeted were based on temperature data from the lake collected year-round. However, because of constraints on temperature control in our lab space, our highest temperature tested turned out to be 22°C on average (see Appendix A). Lake Piru regularly reaches 24-25°C at the hottest times of the year (July-September). Because molluscicides usually work better at higher temperatures, which is supported by our current data, mortality rates at 22°C can be considered conservative for the hottest time of the year. However, because eradication is the ultimate goal and scaling up from bioboxes to a lake-wide treatment may present extra challenges, we think it is best to start with a conservative estimate for how much molluscicide it will take to achieve eradication. On the other hand, we do want to minimize the effective amount of product in order to minimize any potential impacts to nontarget species so we think the 22°C results are a good starting point for planning purposes.

The results suggest that it will take less product and time to apply an effective dose of the copper than the potassium chloride at any given temperature. However, the copper may require “bumps” in concentration depending on how fast the dcu decreases in the water column. Bumps were simulated by refreshes in this study. The next step for optimizing copper treatments against quagga mussels at Lake Piru will be to experiment with how fast the dcu decreases and determine what the fewest number of required bumps will be for an effective treatment at different temperatures.

Appendix A. Summary of Experiments

Round	Target Temp	Actual Temp (mean ± standard deviation)	Start Date	End Date	Treatments	Controls	Mussel Source	Number of Mussels	Biomass Range
Round 1a	10°C	9.60°C ± 1.97	2/1/17	2/28/17	Potash - 150, 200, 250 ppm w/recovery No refresh	Raw lake water No refresh	Creek	Controls = 50/biobox Potash = 100/biobox	*Not recorded* Estimated: 10 g for controls and 20 g for potash per biobox
Round 1b	10°C	10.50°C ±1.82	3/10/17	4/6/17	Copper 60, 120, 180 ppb w/refresh (days 3,6,9,12,17,21,25)	Raw lake water w/ refresh (day 22)	Creek	100/biobox	20-21 g
Round 2	18°C	16.69°C ±2.32	4/28/17	5/26/17	Potash - 150, 200, 250 ppm w/recovery No refresh Copper - 60, 120, 180 ppb w/refresh (days 3,6,9,12,16,20,24)	Raw lake water w/refresh (days 4,7,10,13,16,20,24)	Creek and Lake (50:50)	100/biobox	46-56 g (lake mussels tend to be larger, hence the higher biomass compared to Round 1b)
Round 3	25°C	22.00°C ±1.27	7/6/17	8/15/17	Potash - 150, 200, 250 ppm w/recovery w/ refresh (days 4,7,10,13) Copper - 60, 120, 180 ppb w/refresh (days 3,6,9,13,17,21,25,29,33,38)	Raw lake water w/refresh (days 4,7,10,13,16,19,22,26,30)	Creek and Lake (50:50)	100/biobox	46-56 g (lake mussels tend to be larger, hence the higher biomass compared to Round 1b)