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Research Article

Effects of temperature on mortality of quagga mussels (*Dreissena bugensis*) exposed to potassium chloride and copper-based molluscicides in high conductivity waters

Michael T. Booth[1,2] and Katherine L. Ayres[2]

[1]*University of Cincinnati, Department of Biological Sciences, Rieveschl Hall 820D, PO Box 210006, Cincinnati, OH 45221-0006, USA*
[2]*Stillwater Sciences, 279 Cousteau Place, Suite 400, Davis, CA 95618, USA*

Corresponding author: Michael T. Booth (michael.booth@uc.edu)

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Abstract

Dreissenid mussels (quagga and zebra mussels, *Dreissena bugensis* and *polymorpha*) continue to spread throughout inland waterbodies of North America. Although there are considerable management and mitigation efforts underway to prevent infestation of new waterbodies, there have been relatively few successful efforts to eradicate mussels once detected due to a lack of cost-effective treatments. Two potentially cost-effective molluscicidal agents, potassium chloride (KCl, also called muriate of potash) and copper (formulated as Earthtec QZ®), have successfully treated infested waters, but have varying efficacy under different water quality and temperature conditions, and to date have only been applied in moderate conductivity waters (defined here as < 400 µS/cm) common throughout North America. Natural resource managers need information on the efficacy of these chemicals in high conductivity waters more typical of riverine systems and arid climates, in addition to the range of water temperatures typically encountered in the field. We evaluated the dose-response of quagga mussels to KCl and Earthtec QZ® in high conductivity water (≈ 700–1,400 µS/cm) across a range of temperatures (10, 18, 22 °C) to determine appropriate doses and treatment duration resulting in 100% mortality. Our data indicate that treatments in cool temperatures (i.e., 10 °C) may be challenging for eradication efforts in high conductivity waters because none of the KCl treatments and only one copper treatment resulted in 100% mortality within the experimental period. However, both KCl (> 200 ppm) and copper (120 and 180 ppb) were consistently able to induce 100% mortality in quagga mussels in warmer temperatures (i.e., ≥ 18 °C) and thus may be good candidates for field scale application in high conductivity waters. The results of this study indicate that field-scale application should strongly consider environmental conditions during the period of application when selecting dosages and treatment duration.

Key words: quagga mussel, dreissenid, control, thermal, molluscicide, potash, Earthtec QZ

Introduction

Dreissenid mussels (zebra mussel, *Dreissena polymorpha* [Pallas, 1771] and quagga mussel, *Dreissena bugensis* [Andrusov, 1897]) have spread and continue to spread from their native range in the Caspian and Black Sea drainage basins via anthropogenic modification and conveyance of water systems or transport via watercraft (Karatayev et al. 2007). These mussels often reproduce rapidly and attain high densities that result in fundamental changes to physical, chemical, and biological components of aquatic ecosystems (Nalepa 2010) and cause > $1 billion/year in damage and control costs due to biofouling of water infrastructure (Pimentel et al. 2005). Despite efforts to educate boaters and prevent the movement of nuisance species (Mangin 2001), the spread of dreissenid mussels remains a significant risk to freshwater systems (Dalton and Cottrell 2013). Once established, these mussels have proved difficult to eradicate and limited tools are available for cost-effective treatment that minimizes non-target ecological impacts (Wong and Gerstenberger 2011).

The development and use of molluscicidal agents to control or eradicate dreissenid mussels continues to evolve. Since a primary impact of dreissenid mussels is biofouling of water conveyance structures, many control efforts have focused on small-scale control within infrastructure using molluscicidal chemicals (e.g., chlorine, copper sulfate, quaternary ammonium; Brady et al. 1996; Britton and Dingman 2011; Lake-Thompson and Hofmann 2019) or by more traditional methods like antifouling coatings (Glomski 2015). Substantially more research has focused on zebra mussels, with more limited studies on quagga mussels (Karatayev et al. 2015). Despite considerable interest from the natural resource management community, relatively few field-scale eradication attempts have been reported (e.g., Fernald and Watson 2014; Hammond and Ferris 2019) with varying degrees of success (e.g., Lund et al. 2018).

To the best of our knowledge, all but one field-scale eradication effort (Hammond and Ferris 2019) have targeted zebra, rather than quagga mussels. Luoma et al. (2018) suggest that limited success at the field scale may be partially due to water temperature at the time of application. In a laboratory setting, increased temperature can increase the sensitivity of zebra mussels to toxicants due to increased filtration and metabolic rates (Rao and Khan 2000; Costa et al. 2008; Luoma et al. 2018). Although we expect quagga mussel metabolism will respond similarly to changing temperature, we are not aware of any studies that have examined temperature-dependent mortality in quagga mussels. In addition, since water temperature can vary dramatically among seasons and with lake or reservoir depth, understanding the interaction of toxicity and temperature is essential to increase the success of future eradication efforts for both species.

Most toxicity trials for dreissenid mussels have been conducted in moderate conductivity waters because the North American invasion began in the Laurentian Great Lakes region (specific conductivity ≈ 300 µS/cm; e.g., Costa et al. 2011; Luoma et al. 2018; Lake-Thompson and Hofmann 2019). However, more recent work in the Colorado River (specific conductivity ≈ 1000 µS/cm; Moffitt et al. 2016) found that a much higher dose of potassium chloride (KCl) was required in the higher conductivity water to achieve similar efficacy. Moffitt et al. (2016) also demonstrated that artificially increasing conductivity of low conductivity water (by adding NaCl) reduced mussel mortality. Given the observed variation in effectiveness of molluscicides under differing water quality conditions, further study is required to determine appropriate dosages and treatment durations for a broader range of field conditions.

To date, only two compounds, KCl (also called muriate of potash) and copper (formulated as Earthtec® QZ, a U.S. EPA-registered molluscicide) have been applied successfully at the field scale to eradicate dreissenid mussels in an open water body. Exposure of adult mussels to elevated levels of K^+ leads to shell gaping, soft tissue immobilization, and other physiological responses, including eventual death (Wildridge et al. 1998), but appears to have minimal non-target impacts. Exposure to copper is acutely toxic to mussels (and other taxa) even at relatively low dosages, however mussels can avoid exposure by closing their valves (Kennedy et al. 2006).

In moderate conductivity water (≈ 300 µS/cm; Luoma et al. 2018), both compounds exhibited reduced effectiveness at temperatures below 12 °C and required at least two weeks of treatment to achieve effective eradication. In warmer waters (i.e., ≥ 17 °C), effectiveness was increased and mortality occurred within 4 days. We are not aware of any studies that have investigated the dose and duration relationship for quagga mussels in high conductivity waters.

Our objective in this study was to identify molluscicide dosages, application times, and interactions with water temperature and conductivity to inform control and eradication strategies for quagga mussels at Lake Piru, a high conductivity reservoir (≈ 700–1,400 µS/cm) in Ventura County, California with seasonal temperature changes and stratification (Booth and Culver 2023). Water quality conditions in Lake Piru are considered suitable for quagga mussels, with calcium levels ranging from 57 to 110 mg/L, pH from 7.9 to 8.8, and salinity less than 5 ng/L (United Water Conservation District 2017). Lake Piru is managed by the United Water Conservation District, a groundwater management agency, for the storage and subsequent release of winter precipitation to recharge downstream aquifers. Specifically, we tested KCl and EarthTec® QZ at three product concentrations and three target water temperatures (10, 18, 22 °C) to identify minimum successful dosages, application times, and interactions with local water chemistry that would influence application methods at the field scale. In addition, we sought to assess the feasibility of application by estimating the relative total mass/volume of chemical required at the whole-lake scale.

Materials and methods

General study design

We tested two molluscicides, potassium chloride (KCl, Alpha Chemicals) and copper (copper sulfate pentahydrate, an acid-stablized ionic copper compound; EarthTec® QZ, Earth Science Laboratories, Bentonville, AR) over

Table 1. Experimental parameters and conditions for exposure of quagga mussels to potassium chloride (KCl, muriate of potash) and copper (Cu^{2+}, Earthtec QZ formulation). Baseline specific conductivity (µS/cm at 25 °C) refers to the measured conductivity in raw lake water prior to the addition of the treatment chemicals.

Experiment	Target Temp (°C)	Actual Water Temp (mean ± SD)	Baseline Specific Conductivity (µS/cm, mean ± SD)	Start Date	End Date	Duration (days)	Treatment Refresh	Mussel Source	Number of Mussels per Biobox	Biomass Range (g)
Controls										
1a	10	9.2 ± 1.2	1,553 ± 61	1/29/17	2/25/17	27	No refresh	Piru Creek	50	10
1b	10	10.2 ± 1.7	1,003 ± 17	3/10/17	4/6/17	27	Day 22	Piru Creek	100	20–21
2a	18	16.4 ± 1.7	748 ± 65	4/28/17	5/26/17	28	3 days	Piru Creek: Lake Piru (50:50)	100	46–56
3a	22	21.7 ± 1.1	957 ± 127	7/6/17	8/15/17	40	3–4 days	Piru Creek: Lake Piru (50:50)	100	46–56
3b	18	16.1 ± 2.5	1,301 ± 172	9/18/17	11/20/17	63	3–4 days	Piru Creek	360	46–56
4	10	10.9 ± 1.8	1,295 ± 15	12/14/18	1/11/19	28	3–4 days	Piru Creek	100	46–56
Potassium chloride (KCl) – dosages: 150, 200, 250 ppm										
1a	10	9.1 ± 1.1	1,553 ± 61	1/29/17	2/25/17	27	No refresh	Piru Creek	100	20
2a	18	16.1 ± 1.4	748 ± 65	4/28/17	5/26/17	20	No refresh	Piru Creek: Lake Piru (50:50)	100	46–56
3a	22	22.8 ± 0.7	957 ± 127	7/6/17	7/17/17	11	3 days	Piru Creek: Lake Piru (50:50))	100	46–56
3b	18	15.8 ± 1.8	1,301 ± 172	9/18/17	11/20/17	63	4–7 days	Piru Creek	360	46–56
4	10	11.1 ± 2	1,295 ± 15	12/6/18	1/8/19	33	3 days	Piru Creek	100	46–56
Copper (Cu^{2+}, EarthtecQZ® formulation) – dosages: 60, 120, 180 ppb										
1a	10	9.6 ± 1.4	1,553 ± 61	1/29/17	2/25/17	27	No refresh	Piru Creek	100	20
1b	10	10.6 ± 2.1	1,003 ± 17	3/10/17	4/6/17	27	3 days	Piru Creek	100	20–21
2a	18	16.1 ± 1.5	748 ± 65	4/28/17	5/26/17	28	3 days	Piru Creek: Lake Piru (50:50)	100	46–56
3a	22	21.9 ± 1.1	957 ± 127	7/6/17	8/15/17	40	3–4 days	Piru Creek: Lake Piru (50:50)	100	46–56
4	10	10.5 ± 1.7	1,295 ± 15	12/14/18	1/11/19	28	3–4 days	Piru Creek	100	46–56

a range of water temperatures (10, 18, and 22 °C) to emulate a range of potential application scenarios in temperate lakes. Both compounds have been applied in laboratory and field conditions to control quagga mussel populations. We tested multiple dosages of these two molluscicides (150, 200, 250 parts per million [ppm] K+; 60, 120, 180 parts per billion [ppb] Cu^{2+}) to assess how dose effectiveness varied with temperature in a factorial design. Controls were tested alongside each treatment and contained mussels treated with the same methods but kept in raw lake water with no molluscicide added. We performed two additional experiments relevant to field-scale application: 1) a single dose treatment of copper at 10 °C to test the potential for a single dose application of copper, and 2) potash at 18 °C under substantially different baseline conductivity conditions (≈ 750 versus 1,250 µS/cm). Controls were shared within experimental periods (summarized in Table 1) and not all experimental periods included treatments with both molluscicidal compounds due to space and logistical considerations in the lab facility.

We performed all experiments in a temperature-controlled mobile laboratory located immediately downstream of Santa Felicia Dam in Piru, California.

Unfiltered lake water was sourced from the Santa Felicia Dam penstock for all experiments. We conducted experiments from February to November 2017, with one additional experiment (KCl, 10 °C) conducted in December 2018 to January 2019.

Lake Piru was invaded by quagga mussels in December 2014 and since then mussels have established in the reservoir and in Piru Creek downstream of the dam (Booth and Culver 2023). Quagga mussels were collected from Lake Piru (upstream of the dam) or Piru Creek downstream of Santa Felicia dam, or both, depending on availability. Mussels were acclimated to the test temperature in aerated raw lake water for at least 48 hours prior to each experiment. We housed experimental mussels in replicate "bioboxes" (n = 4), consisting of a 12-litre plastic cooler filled with lake water (4 replicates × 3 dosages = 12 per treatment). Each biobox was aerated for the duration of the experiments. Water was replaced every 3–4 days to replenish food and to remove waste (except during specific experiments noted in Table 1).

For each experimental period, water temperature was controlled by ambient air temperature in the mobile laboratory, which used a modified air conditioning system to cool the room. We targeted three water temperatures (10, 18, and 22 °C) for experimental treatments within the typical range observed in Lake Piru throughout the year (9.3 to 26.3 °C). Since water temperature was indirectly controlled, treatment temperatures fluctuated somewhat over the course of the experiment, but average temperature was within 1°C of target temperature for 10 and 22 °C targets, and 3 °C for the 18 °C target (Table 1). Water temperatures in bioboxes at opposite sides of the laboratory were recorded at 1-hour intervals throughout the experiments using at least two HOBO pendant temperature loggers (Onset, Bourne, MA). Water quality (temperature, dissolved oxygen, pH, conductivity) was monitored every 1 to 3 days using a Quanta multiparameter water quality sensor (Hydrolab, Austin, TX) or PocketPro CondLR meter (Hach, Loveland, CO).

Molluscicides were batch-mixed (one batch per dosage) in designated plastic bins with lake water at the beginning of each experiment and immediately prior to each water change. Raw lake water was stored in buckets in the laboratory prior to each water change to minimize temperature fluctuations during water changes. KCl treatment concentration (target dosage: 150, 200, 250 ppm) was measured by weight added per volume water, and relative concentration monitored via specific conductivity between water changes and every 1 to 3 days in the bioboxes. Although the KCl dose was replaced during water changes, we did not observe changes in conductivity indicating changes in the availability of K^+. Background potassium levels were not measured, but reported levels in Piru Creek (USGS gage #11109800), the source water for our study, were approximately 3–4 ppm in 1992.

For each copper dosage level, copper concentration (target dosage: 60, 120, 180 ppb Cu^{2+}) was measured every 1 to 3 days in one randomly selected replicate biobox (three bioboxes total) immediately prior to and following a water change. Because the bioboxes were filled from the same batch of copper solution for each dosage and the biomass of mussels was similar, the other three replicate bioboxes were assumed to be similar in copper concentrations. Except in the "no refresh" experiment, copper was refreshed with each water change to simulate replenishment ("bump") treatments performed in field applications (e.g., Lund et al. 2018; Hammond and Ferris 2019) to return experimental dosages to the target levels. Copper concentrations were measured using the Copper Porphyrin protocol (Hach method #8143) using a DR 900 Multiparameter Handheld Colorimeter (Hach, Loveland, CO). The sample cells used for the protocol were soaked in a 10% nitric acid bath about once every two weeks to remove any buildup on the inside of the glass. Raw lake water in Lake Piru was measured to have a background concentration of 4 ppb copper at the beginning of the experiments.

For each treatment (KCl, copper, and controls) and dosage (high, medium, low, control), we selected approximately 400 acclimated live mussels of various sizes over 5 mm, dried them to remove excess water, and bulk weighed (target: 46 to 56 g wet weight). Mussels < 5 mm were not included in the experiment because they were difficult to score for mortality (see methods below). The mussels were divided into four groups (≈ 100 mussels per replicate biobox), placed in mesh bag(s), and suspended in the bioboxes. For KCl experiments, mussels were evenly divided into bags containing ≈ 10–20 mussels (10–18 bags total) to facilitate a necessary "recovery" step explained further in the *Mortality* section. For copper experiments, all mussels were housed in a single bag. Mesh bags were hung so that the bag and all the mussels were under water but not touching the bottom of the cooler. In early experiments, mussels were observed to "climb" up the bags using their byssal threads, therefore bags were checked regularly and adjusted as needed to make sure they were hanging low enough in the water to prevent mussels from climbing out of the water and decreasing their exposure to the treatment. During one experiment (18 °C, KCl, September 2017) large mussels were unavailable due to a population crash in the lake, so we used an equivalent biomass, but higher number of mussels (≈ 360 individuals/biobox, ≈ 20 mussels/bag). Experiment duration ranged from 11–33 days (KCl) or 27–40 days (copper). The KCl experimental duration was shorter because sampling frequency was limited by the total number of bags (i.e., mussels were subdivided into groups of 10 and a random group was sampled without replacement at each time interval over the course of the experiment). This sampling approach was needed to facilitate the recovery step explained further below.

Mortality

We scored mussels for mortality every 1–3 days depending on temperature (i.e., every 3 days at 10 °C and daily at 22 °C). Mussels were scored as "dead" if they were 1) "gaping" with shell open and the shell remained open after jostling the mesh bag, gently prodding with a probe, or the shell popped back open when the researcher closed it, 2) shells popped open easily with very gentle prodding with a small probe or a fingernail, or 3) the meat (fleshy part of the mussel) was missing from the shell. Mussels were considered alive if the shell closed after jostling or gentle prodding with a probe or the shell remained firmly closed despite gentle prodding. For copper treatments and controls, dead mussels were removed and disposed of through on-site burial according to United Water Conservation District's *Quagga Mussel Monitoring and Control Plan* and live mussels were returned to the bag and placed back in the treatment biobox. Clear symptoms of mortality were difficult to establish for KCl-treated mussels (some mussels temporarily gape while exposed to KCl, but they can recover if removed from exposure (R. Claudi 2017 *pers. comm.*). Therefore, we implemented a "recovery" step for KCl treatment. Controls for Experiment 3b also went through the recovery step (Table 1). Similar to Moffitt et al. (2016), recovery consisted of selecting a random bag of mussels from each replicate KCl biobox at each assessment interval, rinsing the bag in raw lake water at temperature, and then suspending the bag in the isolated "recovery" bucket with fresh, raw lake water and an aerator for at least 48 hours. After the recovery step, mortality was assessed as described above, and scoring was considered to correspond with the day mussels were initially removed from the treatment biobox. There were separate recovery buckets for controls and KCl treatment bags.

Statistical analyses

Following Claudi et al. (2013), we fit a three-parameter binomial log-logistic dose-response model (LL.3) using the drm function from the drc package (Ritz et al. 2015) in Program R (R Core Team 2022) of the following functional form:

$$p = \frac{d - 0}{1 + \exp\left(b \times (\log(x) - \log(e))\right)}$$

where p is the proportion of mussels found dead at a certain time of exposure, and the fitted parameter d is the maximum proportion of dead mussels reached, b is the scale parameter that determined the shape of the dose-response curve, x is exposure time, and e is the exposure time that resulted in 50% of the maximum effect observed (d) in a treatment group. All parameters of this model were allowed to vary among the dosages within each treatment, however, the parameter d was constrained using the drm arguments "lowerl" and "upperl", which were set to 0 and 1, respectively. Although model-fits were computationally possible for most experiments, some parameter estimates are suspect and exceed reasonable bounds. We interpreted values for d greater than 1.1 and e greater than the experiment duration as dose-response models that did not asymptote within the experimental period. We calculated the dose duration for 99% mortality using the ED function from the drc package for model fits with significant ($p < 0.05$) parameter estimates and values for $d \geq 0.99$.

Application comparison at field scale

Although bioboxes may not reflect all environmental conditions that could influence field scale application, they can provide a relative comparison and order of magnitude estimate to consider when scaling up product volumes to a larger area, such as a full lake treatment. To estimate one example of relative quantities required for application at the whole lake scale for Lake Piru, we assumed a lake volume of 2.47×10^{10} L and minimal lake inflows, which is approximately 25% of the lake capacity – the typical lake conditions during the drought period 2014 to 2019. We assumed that KCl would require a "single" application (though this application might be spread over an extended period due to logistical considerations) because KCl is not consumed by organisms and remains available in the water body over extended periods. Copper would require replenishment applications to maintain the effective dosage (every three days) because copper is consumed by biological organisms and available copper decreases over time. The treatment duration was the mean 99% exposure duration observed during an experiment that resulted in ≈ 100% mortality. We assumed that the chemicals would be delivered to the site in standard capacity semi-truck trailers (20-ton dump trailer – 18,143-kg or 26,000-L tank trailer). Because deployment of these products is site-specific (e.g., Fernald and Watson 2014; Lund et al. 2018; Hammond and Ferris 2019) and has not been completed at a scale similar to Lake Piru, we did not estimate application time. However, we assume that storage of the chemicals and the logistics of their application will differ substantially and used cost estimates from Hammond and Ferris (2019).

Results

Water chemistry

Within each experiment, water quality parameters were similar among replicate bioboxes and within the environmental tolerances reported for quagga mussels (Garton et al. 2014). Dissolved oxygen levels were > 95% saturation and 8–10 mg/L, except during Experiment 4, when mean DO % saturation was ≈ 70% and 7.8 mg/L. pH ranged from 7.7 to 8.5. Due to changes

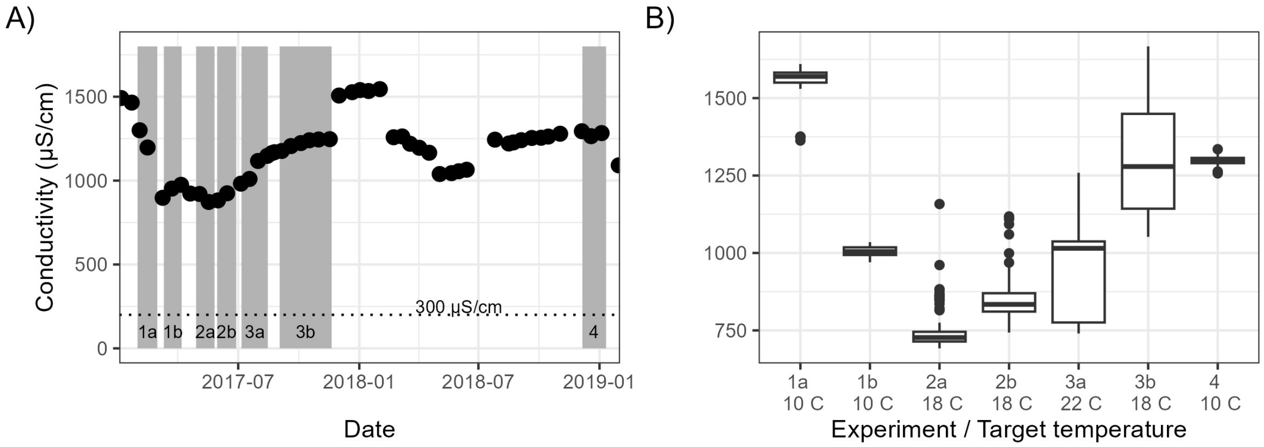

Figure 1. Variation in specific conductivity in A) Lake Piru and B) control bioboxes within experimental periods. Specific conductivity from moderate conductivity Lake Ontario and Minnesota lakes (≈ 300 µS/cm; Moffitt et al. 2016; Luoma et al. 2018) is provided for reference.

in inflows to Lake Piru (local rainfall in February 2017 and 2018, as well as an out-of-basin water delivery from the California State Water project in December 2017) and evaporation from the lake surface, specific conductivity changed over the course of the experiments (Figure 1), ranging from 692 (Experiment 2a) to 1,667 µS/cm (Experiment 1a). However, specific conductivity was consistently higher than reported for mussel toxicity trials in Lake Ontario water (≈ 300 µS/cm; Moffitt et al. 2016).

Potassium chloride

For low temperature experiments (10 °C), the mean temperature was approximately 1 °C cooler (Experiment 1a) and 1 °C warmer (Experiment 4) than the target temperature of 10 °C. Mussel mortality was variable within and among experiments (Figure 2A). Experiments 1a and 4 differed in several ways (Table 1): 1a did not receive water refreshes, had 50% less biomass than Experiment 4, mean specific conductivity was approximately 260 µS/cm higher in 1a ($p < 0.001$), and control mortality was 15% higher in Experiment 4 due to high initial mortality at the beginning of the experiment (Figure 2A). In Experiment 1a, estimated total mortality (*d*) did not exceed 50% for any dosage tested (Supplementary material Appendix 1: Fitted model parameters for dose response models – NA values indicate the model did not asymptote). In Experiment 4, total mortality (*d*) was ≈ 100% for the 200 and 250 ppm dosages, but the model-estimated 99% effective exposure durations were > 20 days longer than the experimental periods (27 [Experiment 1a] and 30 [Experiment 4] days) and should be interpreted with caution.

For medium temperature experiments (18 °C), the mean temperature was approximately 2 °C cooler (experiments 2a and 3b) than the target temperature of 18 °C (Table 1). Experiment 2a was conducted when lake water conductivity was 550 µS/cm lower than 3b ($p < 0.001$). Mussel mortality was ≈ 100% for 200 and 250 ppm dosages in Experiment 2a (Figure 2B), and

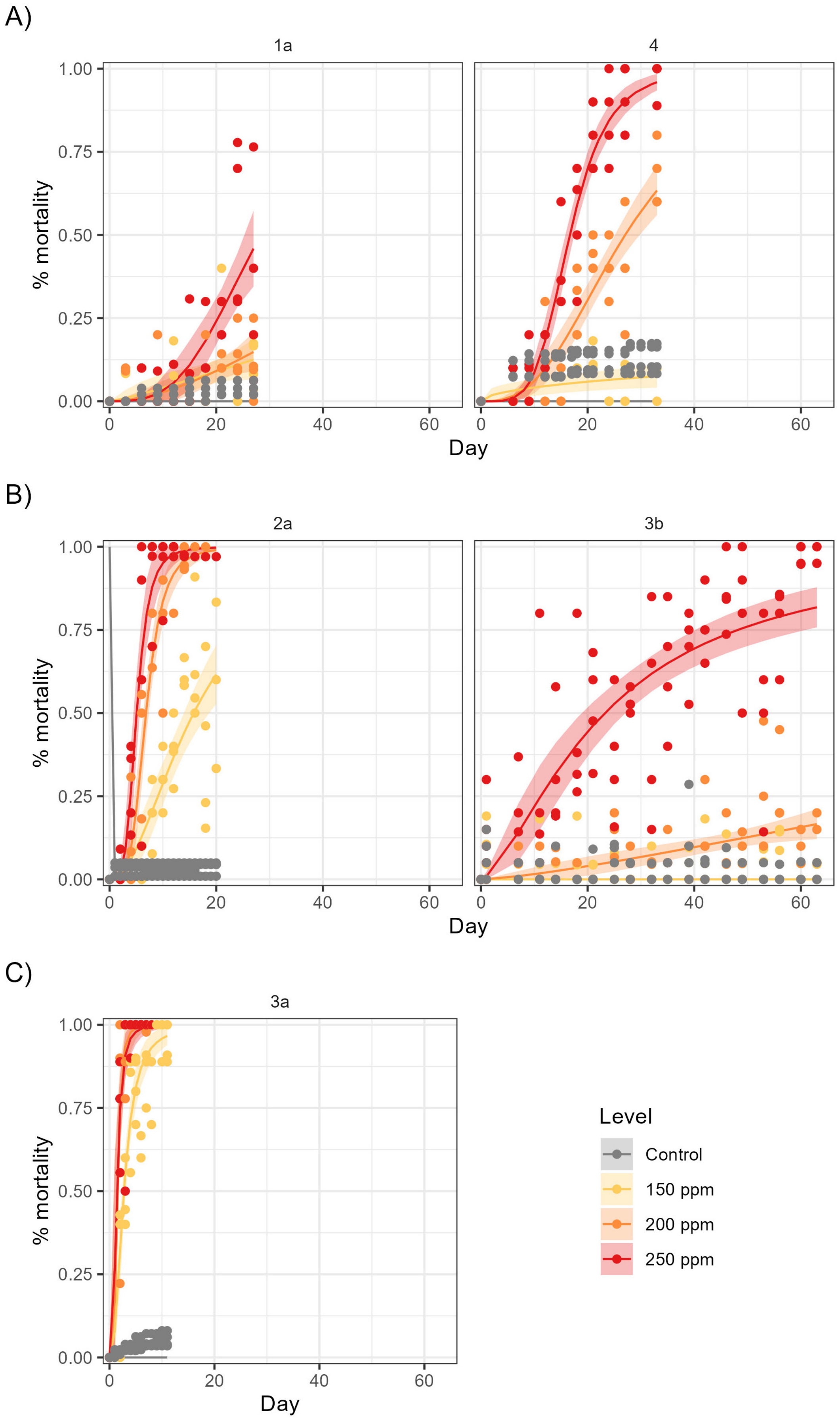

Figure 2. Measured mortality for adult mussels exposed to KCl at A) 10 °C, B) 18 °C, and C) 22 °C with log-logistic dose-response model fits. Colored bands are 95% confidence intervals and points are mortality values from replicate bioboxes.

the model-estimated 99% effective exposure duration was 21.6 and 14.4 days, respectively, with 50% mortality occurring in less than 7 days for both dosages (Appendix 1). Conversely, in the higher background conductivity conditions of Experiment 3b, none of the tested dosage resulted in 100% mortality despite extended exposure time (63 days) and within each dosage, among-replicate variability was high (up to 50% difference in mortality). Dose-response models did not asymptote within the experiment durations for the 18 °C target water temperature.

For high temperature experiments (22 °C), the mean temperature was about 0.8 °C (Experiment 3a) warmer than the target temperature of 22 °C (Table 1). Mussel mortality was ≈ 100% for all dosages and occurred rapidly (Figure 2C), with model-estimated 99% effective exposure duration within the experimental duration (11 days) for 200 and 250 ppm dosages, and 17.7 days for 150 ppm, and 50% mortality (*e*) in less than 2 days for all dosages (Appendix 1).

Copper – refresh

The mean temperature for experiments 1b and 4 were about 0.5 °C warmer than the target temperature of 10 °C but within one standard deviation of the mean (Table 1). Experiment 1b and 4 differed in several ways (Table 1): 1b had 50% less biomass than Experiment 4 and mean specific conductivity was about 290 μS/cm lower in 1b ($p < 0.001$). In Experiment 1b, total mortality (*d*) was ≈ 100% for the 120 and 180 ppb dosages (Figure 3A), but only ≈ 15% for 60 ppb (Figures 3A, 4A). 50% mortality was achieved within 9 to 6.5 days for the 120 and 180 ppb dosages, respectively. In Experiment 4, total mortality (*d*) was ≈ 96% for 180 ppb, did not asymptote for 120 ppb, and was ≈ 17% for 60 ppb (Figures 3C, 4A). Copper concentrations fluctuated dramatically in Experiment 4 (Figures 3D, 4B) relative to Experiment 1b, and the mean dose over the treatment duration was 50 ppb lower than the 180 ppb target and 20 ppb lower than the 120 ppb target (Table 2).

The mean temperature for Experiment 2a was 2 °C cooler than the target temperature of 18 °C but the target temperature was within one standard deviation from the mean (Table 1). Mussel mortality was ≈ 100% for 120 and 180 ppb dosages in Experiment 2a (Figure 4C), and the model-estimated 99% effective exposure duration was 31.9 and 11.4 days, respectively, and 50% mortality in less than 7 days for both dosages. The 60 ppb treatment did not asymptote. Copper concentrations were less variable than Experiment 4 (target temperature 10 °C), and the mean dose over the treatment duration was only about 5 ppb lower than the target dosages (Table 2, Figure 4D).

The mean temperature for Experiment 3a was the target temperature of 22 °C (Table 1). Mussel mortality was ≈ 100% for 120 and 180 ppb and occurred rapidly (Figure 2C), with model-estimated 99% effective exposure

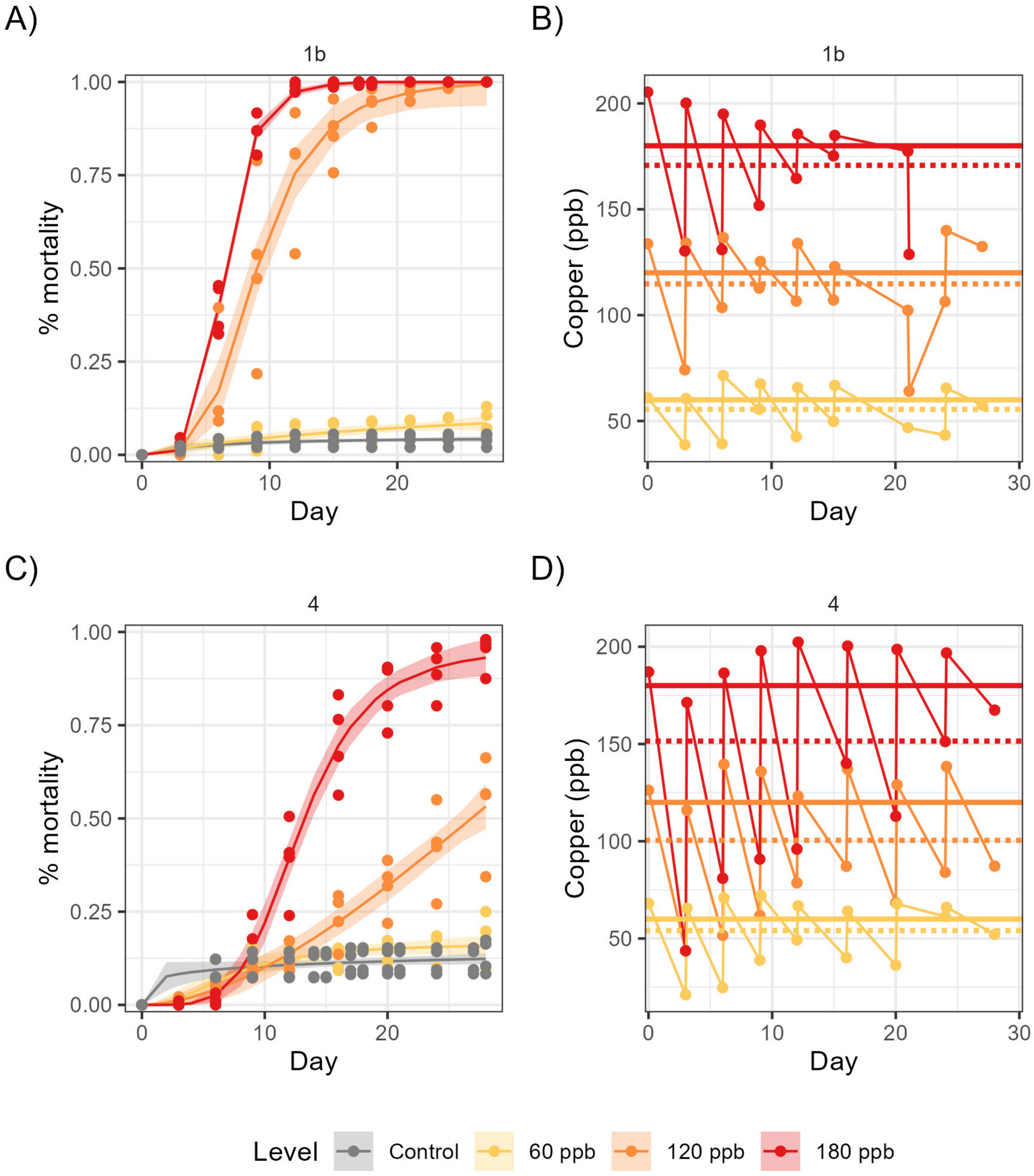

Figure 3. Comparison of measured mortality for adult mussels exposed to copper at 10 °C and copper concentrations over time for Experiment 1b (A), which had 50% less biomass and lower mean specific conductivity than Experiment 4 (C) with log-logistic dose-response model fits. Colored bands are 95% confidence intervals and points are mortality values from replicate bioboxes. Measured copper concentrations in bioboxes for B) Experiment 1b and D) Experiment 4. Solid horizontal lines are target concentrations, dashed horizontal lines are mean concentration over the entire experiment duration.

duration of 7.7 and 5.4 days for 200 and 250 ppm dosages, respectively, and 50% mortality in less than 3 days for both dosages (Appendix 1). The 60 ppb dosage had variable responses, and the fitted dose-response model did not asymptote, but mortality approached 100% by the end of the experiment (day 40). Copper concentrations fluctuated greatly, similar to Experiment 4 (target temperature 10 °C), with the mean dose over the treatment duration

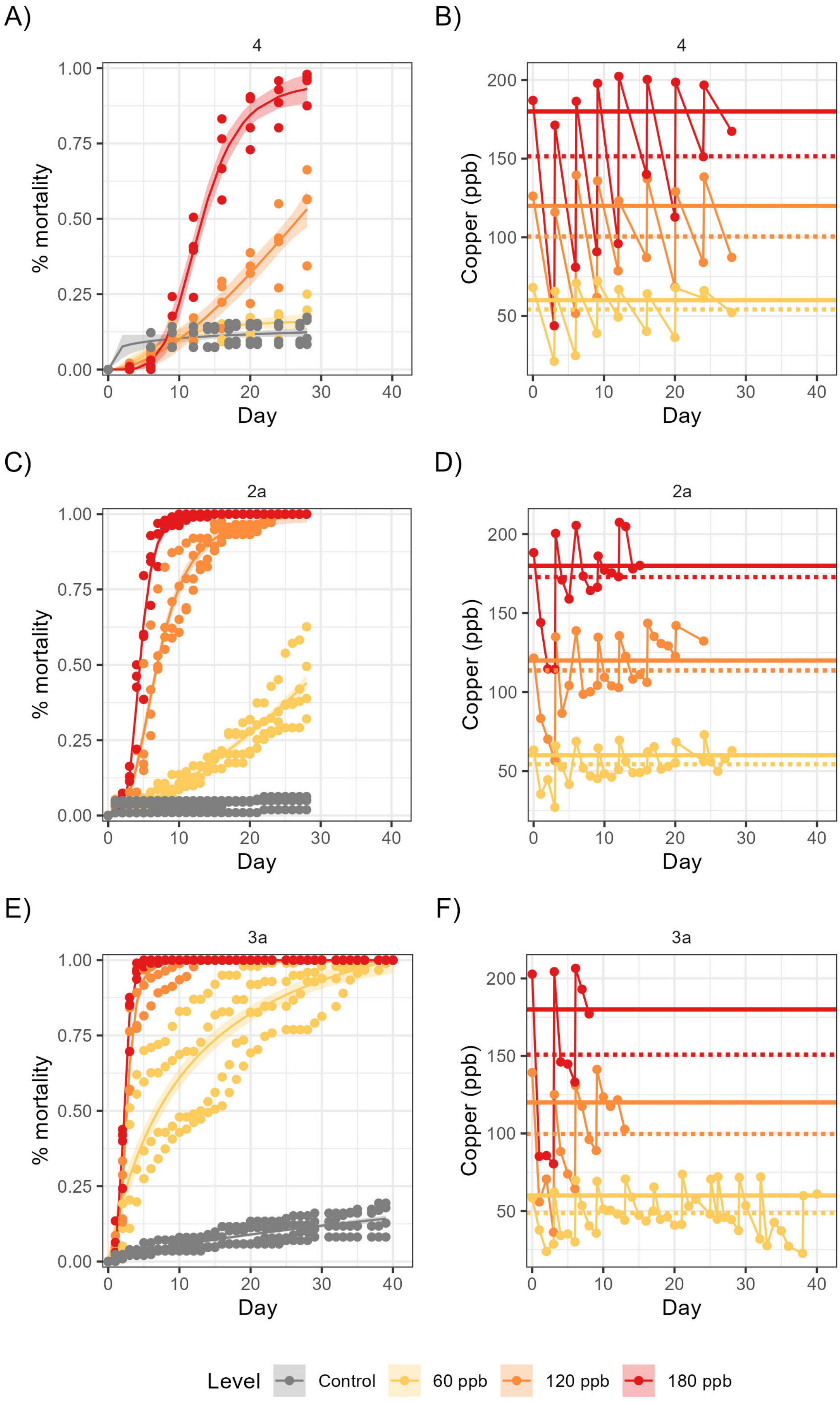

Figure 4. Measured mortality for adult mussels exposed to copper (Earthtec QZ®) at A) 10 °C, C) 18 °C, and E) 22 °C with log-logistic dose-response model fits. Colored bands are 95% confidence intervals and points are mortality values from replicate bioboxes. Measured copper concentrations in bioboxes at B) 10 °C, D) 18 °C, and F) 22 °C. Solid horizontal lines are target concentrations, dashed horizontal lines are mean concentration over the entire experiment duration.

Table 2. Target and mean dosages achieved in copper treatment experiments. Variation in copper concentration over time is shown in Figures 3B, 3D, 4B, 4D, 4F, 5B, and 5D.

Experiment	Target Temperature (°C)	Target Dose (ppb)	Mean dose (ppb)	SE	Target - mean dose (ppb)
1a	10	60	33.6	4.1	26
1a	10	120	62.8	6.8	57
1a	10	180	84.6	8.9	95
1b	10	60	55.5	2.9	5
1b	10	120	114.8	5.6	5
1b	10	180	170.8	7.5	9
2a	18	60	54.3	1.8	6
2a	18	120	113.7	4.3	6
2a	18	180	172.9	6.0	7
3a	22	60	48.7	2.1	11
3a	22	120	99.6	7.6	20
3a	22	180	150.8	15.1	29
4	10	60	54.1	4.2	6
4	10	120	100.5	8.4	20
4	10	180	151.5	12.8	29

50 ppb lower than the 180 ppb target, 20 ppb lower than the 120 ppb target, and 10 ppb lower than the 60 ppb target (Table 2, Figure 4F).

Copper – no refresh

To test the potential for a single dose application of copper, we compared the "no refresh" Experiment 1a to Experiment 1b, where the dosage was reapplied every 3 days until day 12, then every 4–5 days. Both experiments had similar mussel biomass and water temperature (Table 1). However, specific conductivity was about 550 µS/cm lower in Experiment 1b. In Experiment 1a (no refresh), none of the dosages resulted in high mortality, with only 180 ppb resulting in appreciable mortality (d = 0.56). In Experiment 1b (with refresh), total mortality (d) was ≈ 100% for the 120 and 180 ppb dosages (Figure 5C), but only ≈ 15% for 60 ppb (Appendix 1). 50% mortality was achieved within 9 to 6.5 days for the 120 and 180 ppb dosages, respectively. In Experiment 1a, when the copper treatments were not refreshed, copper concentrations continuously declined (Figure 5B, resulting in mean dose over the treatment duration of 95 ppb lower than the 180 ppb target, 57 ppb lower than the 120 ppb target, and 26 ppb lower than the 60 ppb target (Table 2, Figure 5B). In contrast, Experiment 1b, which included refreshed copper treatments, had mean copper doses only 5–9 ppb lower than the target dosages (Table 2, Figure 5D).

Application comparison at field scale

Deployment of these products is site- and condition-specific (e.g., Fernald and Watson 2014; Lund et al. 2018; Hammond and Ferris 2019) and has not been completed at a scale similar to Lake Piru, so we did not estimate application time. However, given the quantities estimated for application, we assume that storage of the chemicals prior to application and the logistics of their application will differ, with KCl requiring both more storage space

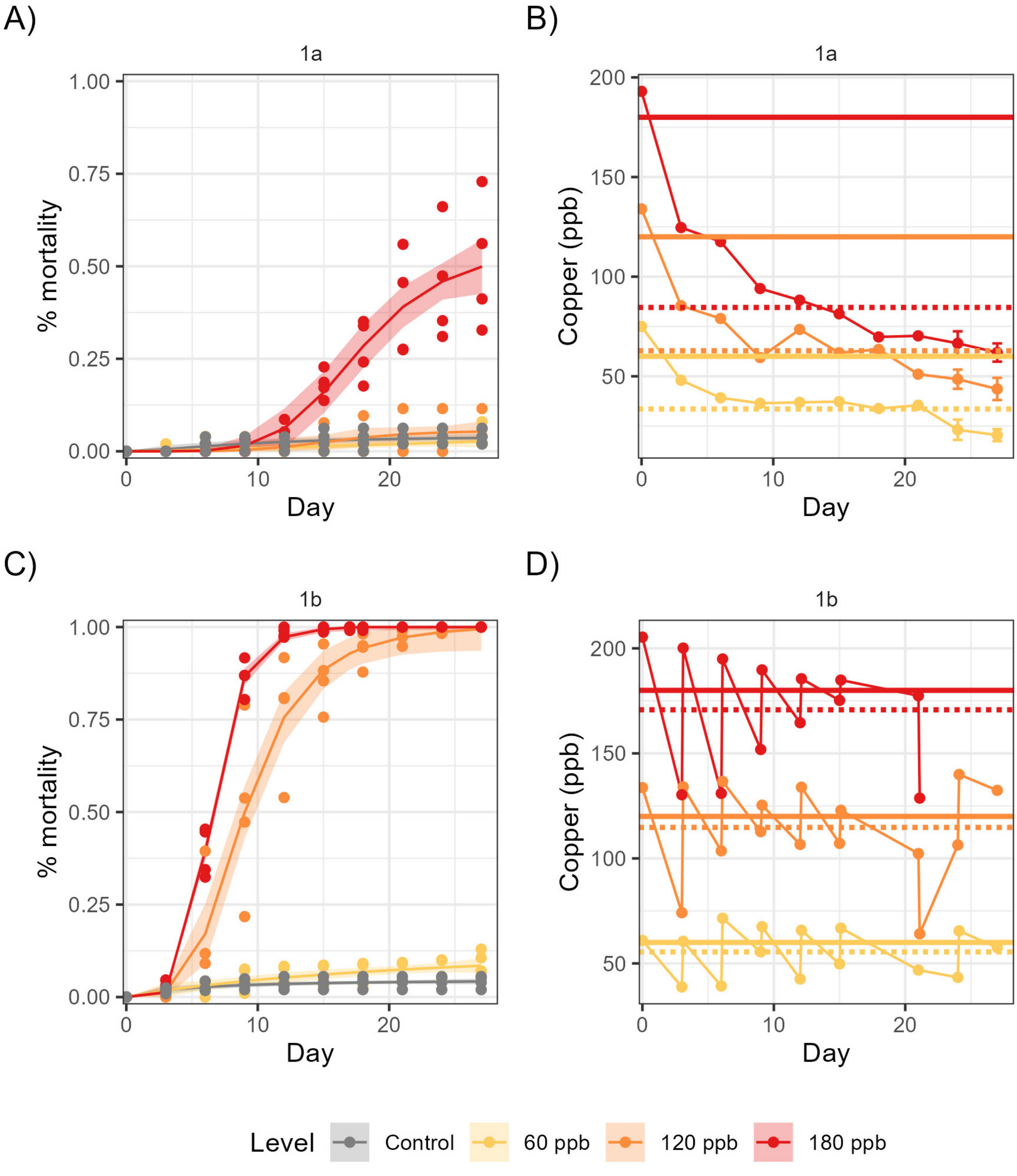

Figure 5. Comparison of measured mortality for adult mussels exposed to copper at 10 °C and copper concentrations over time for Experiment 1a (A), which received only a single dose of copper and Experiment 1b, which included refreshed copper treatments (C) with log-logistic dose-response model fits. Colored bands are 95% confidence intervals and points are mortality values from replicate bioboxes. Measured copper concentrations in bioboxes for B) Experiment 1a (without refresh) and D) Experiment 1b (with refresh). Solid horizontal lines are target concentrations, dashed horizontal lines are mean concentration over the entire experiment duration.

prior to application and greater effort to apply. Hammond and Ferris (2019) estimate that the total cost of copper treatment 70–90% less than KCl treatment.

Based on the experimental results, KCl application was only effective at 18 and 22 °C. Assuming a treatment of 200 to 250 ppm (in a single application),

Table 3. Relative field-scale estimates for treatment of Lake Piru using potassium chloride or copper for whole-lake eradication. Temperature-dosage combinations that did not consistently result in 100% mortality are listed as "mixed" and in these cases, treatment durations is from the experiment which resulted in complete eradication. The total number of treatments for copper is estimated assuming additions every 3 days – however, in an actual application, timing would be based on measured copper levels in the water.

Potassium chloride (KCl)						Copper (Earthtec® QZ)					
Temp (°C)	Mort	Treatment duration (days)	Total treatments	Mass (kg)	Truckloads	Temp (°C)	Mort	Treatment duration (days)	Total treatments	Volume (L)	Truckloads
150 ppm						120 ppb					
10	no	–	–	–		10	mixed	30	11	722,908	28
18	no	–	–	–		18	yes	32	12	788,627	31
22	yes	18	1	3,701,466	205	22	yes	8	4	262,876	11
200 ppm						180 ppb					
10	no	–	–	–		10	mixed	15	6	591,840	23
18	mixed	22	1	4,936,316	273	18	yes	11	5	493,200	19
22	yes	6	1	4,936,316	273	22	yes	6	3	295,920	12
250 ppm											
10	no	–	–	–							
18	yes	15	1	6,165,000	340						
22	yes	10	1	6,165,000	340						

Temp = temperature; Mort = mortality.

the experiment predicts that an effective eradication effort in Lake Piru would require application of 205 to 340 truckloads of KCl (Table 3), depending on the dosage. Under the experimental conditions, copper was not effective at 60 ppb, regardless of temperature. Copper had mixed effectiveness at cold temperatures and required longer treatment periods. At 22 °C, both 120 and 180 ppb treatments would require approximately 1 week of application and 11–12 truckloads of chemical, whereas at 18 °C, the 120 ppb treatment would require one month of application (31 truckloads) and 180 ppb treatment would require 11 days (12 truckloads). Assuming replenishment rates similar to our experimental refresh rates (3 days), the total applied dosage would exceed 1 mg/L copper (the EPA permitted dose permitted in a single treatment) for both 120 ppb and 180 ppb doses at 10 and 18 °C.

Discussion

We found a clear relationship between water temperature and dose-effectiveness for both KCl and copper, with low or variable mortality at cooler temperatures requiring longer exposure durations, and high mortality at high temperatures requiring shorter exposure durations. This relationship indicates that field scale application should strongly consider water temperature during the period of application, particularly regarding dosages and treatment duration. Stratification of temperature, which occurs when bodies of water have differing temperatures near the surface and at depth would be another important consideration for treatment.

Our data indicate that treatments in cool temperatures (i.e., 10 °C, Figures 2A, 4A) may be challenging for eradication efforts in high conductivity waters. None of the potassium chloride treatments resulted in 100%

mortality within the experimental period (similar to the findings of Luoma et al. 2018) – *e* parameters were 1.6 to 4.3 times longer than the experimental periods. Results were mixed for copper, with only one of the two experiments resulting in complete mortality within 27 days of exposure. Both KCl (> 200 ppm) and copper (120 and 180 ppb) were consistently able to induce 100% mortality in quagga mussels in warmer temperatures (i.e., ≥ 18 °C, Figures 2B, C, 4C, E) and thus may be potential candidates for field scale application in high conductivity waters at these higher temperatures.

Large and natural changes in conductivity (Figure 1), like those that occurred between some experimental periods in Lake Piru, may complicate potential application of KCl and copper. Moffitt et al. (2016) found that a much higher dosage of KCl (200 ppm vs 100 ppm) was required to achieve complete mortality in high conductivity (1,080 μS/cm) Colorado River water compared to Lake Ontario (330 μS/cm). Adding NaCl to Lake Ontario water resulted in a dramatic reduction in mortality at the same KCl dosage. Based on these findings, we suspect that the variability in treatment effectiveness (e.g., Experiment 2a versus 3b, Figure 2B) was a result of changes in conductivity. Unfortunately, our experimental design did not provide sufficient replication under differing conditions to test this hypothesis directly. However, the results are suggestive that KCl experiments under the highest conductivity (> 1295 μS/cm; experiments 1a, 3b, 4) had low and variable mortality.

Copper bioavailability (and toxicity) is also known to vary depending on temperature, pH, and the presence of dissolved organic carbon, major cations, major anions, alkalinity, and sulfide (U.S. Environmental Protection Agency 2022). We did not see a clear reduction in toxicity for copper in experiments 1a and 4 (where conductivity was elevated) that could be directly attributed to water quality, rather than experimental conditions. While mortality required longer exposure times in Experiment 4 and was much lower than Experiment 1b, this was more likely due to the higher biomass of mussels (uptake rates were high and mean dose much lower in Experiment 4) than the change in water chemistry. Regardless, it is possible that variation in water chemistry will influence appropriate dosages, though those effects appear to be less dramatic than observed for KCl treatment.

Selection of a molluscicide for field scale application must also consider potential impacts to non-target organisms and the environment. Copper sulfate, the active ingredient in Earthtec® QZ, is considered toxic to many non-target organisms (National Pesticide Information Center 2022), particularly invertebrates and algae. Some fishes, particularly salmonids like rainbow trout, are particularly sensitive to copper, even at low levels (96h LC50 66–108 ug/L; Taylor et al. 2000), potentially leaving little margin of safety at the dosages tested here – however, we are not aware of published toxicity dosages for the Earthtec® QZ formulation of copper. This is particularly relevant in the context of Lake Piru because Piru Creek downstream of the reservoir is critical habitat for the federally-listed endangered Southern California Steelhead (*Oncorhynchus mykiss*), which could be impacted by chemical treatment within the lake. Treatment can also indirectly lead to fish kills due to rapid mass-mortality of aquatic algae and vegetation, which may lead to anoxia in the water column. It is important to note that Hammond and Ferris (2019) did not observe a substantial impact to the fish community (largemouth bass [*Micropterus salmoides*], smallmouth bass [*Micropterus dolomieu*], gizzard shad [*Dorosoma cepedianum*], bluegill sunfish [*Lepomis macrochirus*], channel catfish [*Ictalurus punctatus*], and black crappie [*Pomoxis nigromaculatus*]) in a Pennsylvania quarry treated with Earthtec QZ® and documented that the zooplankton community was similar to pre-treatment conditions one year after application.

In contrast, the KCl dosages tested here were substantially lower than reported KCl 48-h LC50s (≥ 720 mg/L) for channel catfish and rainbow trout, and KCl is relatively selective to mollusks relative to non-target species (Waller et al. 1993). However, KCl remains dissolved in the water post-treatment (Fernald and Watson 2014) and in waterbodies that discharge into flowing waters (i.e., reservoirs), the dissolved constituents would eventually be transported downstream. Piru Creek is a tributary to the upper Santa Clara River, which is impaired by excessive chloride levels (> 80 mg/L). A Total Maximum Daily Load (TMDL) for chloride was established for the upper Santa Clara River watershed (U.S. Environmental Protection Agency 2003) and could potentially influence the feasibility of KCl as a molluscicide in systems with similar impairments.

Considerations for field scale application

Lakes that stratify are likely to have a gradient of temperatures from the surface to the bottom during the stratified period. For example, Lake Piru typically stratifies between March and September, and surface temperatures may be up to 10 °C warmer than temperatures below the thermocline (typically ≈ 10 m depth). Our findings indicate that both KCl and copper are most effective at warm temperatures, but even relatively small differences in temperature (4 °C) can result in substantially different mortality rates and effective exposure time depending on the dosage. So while the stratified period has the warmest surface temperatures and should exhibit high and rapid mortality, mussels in deeper sections of the lake are likely to have substantially lower mortality rates and/or longer required exposure time, even if exposed to an equivalent dosage of molluscicide. For example, in a lake-scale application of Earthtec® QZ, Hammond and Ferris (2019) observed that mussels in the metalimnion (5–10 °C) took up to 40 days to die, while those in surface waters (> 20 °C) died within the first week of exposure.

Both potash and copper appear to be effective molluscicides at or above 18 °C, however, they differ in considerations for application. Potash (KCl) is a generic compound that is widely used in fertilizers and Earthtec® QZ is a proprietary chemical. While KCl may only require a single application and may remain effective as long as it is not diluted, the overall magnitude of chemical estimated to achieve complete mortality in a high conductivity system like Lake Piru is daunting. At a minimum, more than 200 truckloads would be required for application at 150 ppm and 340 truckloads at 250 ppm if the reservoir was at 25% capacity. Muriate of potash commodity prices have been volatile over the 5 year period 2018 to 2023 ranging from US$215 to US$1202 per metric ton (World Bank Commodity Prices). Using these prices, the cost of the product alone for a 150 ppm treatment would be approximately US$795,715–$4,548,600 and a 250 ppm treatment would be about US$1,325,475–$7,410,330. However, the transportation (and potential storage, application, and environmental fate) of this volume of material represents additional significant costs and challenges to the use of potash in a water body the size of Lake Piru.

In contrast, regardless of dosage applied, the volume of copper (as Earthtec® QZ) is an order of magnitude less than potassium chloride – between 3 and 4 truckloads per treatment. While copper was less effective at low temperatures, one of our experiments showed complete mortality for both 120 and 180 ppb treatments in less than one month. As a relative comparison with KCl, we estimated total volume of Earthtec® QZ based on the 3-day refresh rates used in our experiments. However, this may substantially overestimate the total volume of Earthtec® QZ needed if field uptake rates are lower (only 3 applications were required in Hammond and Ferris 2019) and highlights the need for field-scale assessment of copper uptake rates prior to application. Prior work indicates application of Earthtec® QZ can be relatively low cost and effective at reducing or eliminating quagga mussels in smaller systems, with an estimated cost of US$0.06 per m^3 treated (Hammond and Ferris 2019). Assuming similar costs, treatment of Lake Piru with copper would be approximately US$1.5 million.

Authors' contribution

MTB: research conceptualization; sample design and methodology; data analysis and interpretation; manuscript writing. KLA: research conceptualization; sample design and methodology; investigation and data collection; data analysis and interpretation; manuscript data preparation, writing, review, editing.

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Ethics and permits

The authors have complied with national policies governing the humane and ethical treatment of the experimental subjects and are willing to share the original data and materials if so requested. Ethics approval was not required for these experiments. All research pertaining to this article did not require any research permits.

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Supplementary material

The following supplementary material is available for this article:

Appendix 1. Fitted model parameters for binomial log-logistic dose response models and model-estimated exposure duration resulting in 99% mortality.

This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2024/Supplements/MBI_2024_Booth_Ayres_SupplementaryMaterial.pdf