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Research Article

Control of quagga veligers using EarthTec QZ for municipal water supply and impact on non-target organisms

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Abstract

A bench-scale dose-response study evaluated the efficacy of a commercially available copper-based molluscicide known as EarthTec QZ for controlling quagga veligers collected from three Southern California sites impacted by quagga mussels. A variety of concentrations were tested (0, 3, 16.7, 33.4, and 50.1 µL/L of EarthTec QZ or 0, 0.18, 1.0, 2.0, 3.0 mg/L as copper (Cu), respectively) for different durations (0.5, 2, 5, and 24 hours) on quagga mussel veligers. Water from each study site was also used in non-target 96-hour acute toxicity testing of three other species. A suite of water quality parameters was measured to characterize the differences between the study sites. The federal and California secondary maximum contaminant level and action level for copper in drinking water are 1.0 and 1.3 mg/L as Cu, respectively; therefore, results for the 16.7 µL/L EarthTec QZ (1.0 mg/L as Cu) test condition were the main reference point for assessing the efficacy of EarthTec QZ as a feasible treatment strategy. Under this condition, veliger mortality at two study sites, Lake Mathews, and a downstream water treatment plant, was found to be greater than 60% and 85% after 5 hours, and 95% and 100% after 24 hours, respectively. Lower efficacy was observed for the Lake Piru study site, with approximately 30% veliger mortality after 24 hours. Alkalinity, dissolved organic carbon, and chemical oxygen demand were found to be significant variables for veliger mortality in these hard water locations based on linear regression modeling. The three non-target indicator species were adversely affected by EarthTec QZ at concentrations tested. These results indicate that this product has potential as an effective chemical control agent against quagga veligers at low doses, but the aforementioned water quality parameters must be considered with full-scale application to optimize target efficacy, while minimizing exposure to non-target species and costs.

Key words: quagga mussel veliger, copper, water quality, toxicity, molluscicide, bench-scale testing

Introduction

The quagga mussel (*Dreissena rostiformis bugensis* (Andrusov, 1987)) is an invasive species that has negatively impacted aquatic environments throughout North America and Europe. Their tolerance to a wide range of environmental conditions, ability to outcompete native filter-feeding aquatic species for resources, and capacity to quickly reproduce has led to their rapid expansion across the continent (Benson et al. 2022). In addition to adversely impacting native aquatic environments, their economic impact can also be severe. As the mussels establish and infest water bodies, they can clog water supply systems and equipment (i.e., intake and conveyance pipes, pumps, valves, and vessels). Increased maintenance is required once their populations have established, and monitoring and control measures are required to prevent their spread if they cannot be eradicated.

In 2007, quagga mussels were first detected in Lake Mead, Nevada, a reservoir on the Colorado River and the nation's largest freshwater impoundment. Later that same year, they were found in various waterbodies that receive Colorado River water. Quagga mussels have also been found attached to infrastructure in some reservoirs fed by the California State Water Project (SWP) (California Department of Water Resources 2021). It is anticipated that zebra mussel (*Dreissena polymorpha* (Pallas, 1771)) and quagga mussel infestations will be a long-term issue in North America with costs estimated at over $500 million annually in the Pacific northwest region alone (Pacific Northwest Economic Region Foundation and Pacific States Marine Fisheries Commission 2015). As California contains complex water bodies of varying quality used for multiple purposes, the presence of quagga mussels threatens the beneficial uses of reservoirs, rivers, streams, and recharge basins that depend on imported water. The potential for further spread of mussels limits distribution of both local and imported water resources for key uses such as storage, recharge of groundwater aquifers, flood control, and habitat for aquatic species. Under California Fish and Game Code (2010; Section 2302, Chapter 3.5), the California State legislature empowered the California Department of Fish and Wildlife (CDFW) to require water agencies infested with invasive mussels to develop and implement a plan to control or eradicate them within their systems (CDFW 2011).

Physical control measures like desiccation (i.e., complete drying of the area) are effective, but can only be implemented on a small scale. Thus, this mitigation strategy is infeasible for some locations. Chemical methods have been used to control quagga mussels with varying success. Municipal water supply agencies have explored applying conventional oxidants such as sodium hypochlorite (chlorine) to control infestations (CDFW 2011). At low doses, free chlorine is moderately effective for controlling quagga veligers (the larval stage), while also relatively inexpensive and compatible with raw water supplies. However, free chlorine treatments in raw water supplies are limited to low doses to minimize the formation of disinfection by-products (DBPs) like trihalomethanes (THMs) and haloacetic acids (HAAs), a result of chlorine reacting with natural organic matter present in surface waters. These DBPs are carcinogenic, and the United States Environmental Protection Agency (USEPA) has established maximum contaminant levels (MCLs) for them in drinking water. Long-term use of low dose chlorination of water in the Colorado River Aqueduct (CRA) system has not led to large increases in DBPs or MCL exceedances in downstream drinking water supplies (Metropolitan Water District of Southern California 2022), yet this treatment has proven to be insufficient for veliger eradication.

Due to these issues, alternative chemicals have been explored for controlling or eradicating quagga mussels. Studies using copper sulfate products as molluscicides have shown especially promising results. One product, called EarthTec QZ, has been shown to be effective at controlling and eradicating veliger and adult dreissenid mussels (Watters et al. 2013; Claudi et al. 2014; Lake-Thompson and Hoffman 2019; Hurtado et al. 2021). EarthTec QZ is a liquid copper pentahydrate formulation with low pH that maintains copper in solution as cupric ions to increase its bioavailability. It is listed as an NSF 60 certified product that can be applied in potable water systems. Laboratory and pilot scale studies have shown high mortalities of adult and veliger quagga mussels using low concentrations of EarthTec QZ with short exposure durations. The ability to address quagga mussel infestations using low concentrations of copper is critical for treatment in surface waters used as drinking water supplies, considering that copper has a federal and state drinking water secondary MCL value of 1.0 mg/L as copper (Cu) and an action level of 1.3 mg/L as Cu.

Watters et al. (2013) observed 100% mortality of veligers in petri dishes with water from Lake Mead after 30 minutes using 3 µL/L (0.18 mg/L as Cu) of EarthTec QZ. Testing by Claudi et al. (2014) at Davis Dam on the Colorado River used a flow-through experiment, dosing adult quaggas with various concentrations of EarthTec QZ, and observed 99% mortality after 96 hours using 16 µL/L (1 mg/L as Cu). Pilot-scale studies at a drinking water treatment facility on Lake Ontario, Canada, achieved 100% adult quagga mortality in 7 days using 8 µL/L (0.5 mg/L as Cu) EarthTec QZ (Lake-Thompson and Hoffman 2019), and Hurtado et al. (2021) observed 96% mortality of veligers after 10 hours using 4.8 µL/L of EarthTec QZ (0.3 mg/L as Cu). Investigations by Claudi et al. (2016) and Hammond and Ferris (2019) have demonstrated efficacy at low doses (meeting the USEPA drinking water secondary MCL for copper of 1 mg/L) to eradicate and prevent colonization in the Great Lakes region at full-scale lake sites and municipal water treatment plants. Yet a pilot-scale study conducted at Lake Piru, California only achieved 35% veliger mortality after a 24-hour exposure duration using 16 µL/L (1 mg/L as Cu) of EarthTec QZ (Stockton-Fiti 2020), suggesting that longer exposure times may be needed to achieve high rates of veliger mortality, especially at low doses and in certain water chemistries.

Building on this prior work and understanding of available quagga mussel control methods, a bench-scale dose-response study was conducted between December 2019 and July 2021 to evaluate the efficacy of EarthTec QZ for controlling quagga veligers collected from three Southern California sites impacted by quagga mussels. Veliger mortality was assessed at two different temperatures over a range of time and concentration exposures to EarthTec QZ.

Figure 1. Map of study sites (represented by red stars) in Southern California with Los Angeles as the center gray area.

The study objectives were to evaluate the efficacy of various concentrations on veliger mortality rates and the influence of water quality parameters on EarthTec QZ effectiveness. This study also looked at the effect of EarthTec QZ on three non-target species that could be used as indicator species to determine the potential effects of a full-scale field treatment.

Materials and methods

Study sites

Three Southern California locations where quagga mussels were present in surface waters were sampled for this study, Lake Piru, Lake Mathews, and the influent for a water treatment plant (WTP) located on a pipeline downstream of Lake Mathews (Figure 1). Lake Piru, owned and operated by United Water Conservation District (UWCD), captures local storm flows from the Piru Creek watershed with some supplemental imported SWP water from Pyramid Lake. Quagga mussels were initially detected in Lake Piru in 2013, which has since affected UWCD's utilization of water from Lake Piru to recharge several downstream groundwater basins. Sampling at Lake Piru for this study was completed in December 2019; testing was conducted at UWCD's Lake Piru field research lab.

Quagga mussels have impacted the CRA system since they were first discovered in Lake Mead in 2007. Lake Mathews, a Metropolitan Water District of Southern California (MWD) reservoir filled with CRA water in Riverside County, is the source water to many Southern California customers. Raw water exiting Lake Mathews is dosed with chlorine for quagga mussel control as it is delivered into downstream pipelines. The WTP study site represents water from the pipeline after approximately 8 hours of travel time. Correlated sampling was conducted from Lake Mathews and the raw water influent to the downstream WTP study sites in two phases, (a) in September 2020 and (b) June–July 2021. Testing associated with samples collected from these two study sites was completed at the Orange County Water District (OCWD) Field Research Lab in Anaheim, CA.

Veliger sampling and water collection

A Specific Use Scientific Collecting Permit was approved for the study by CDFW prior to commencing any veliger collection. At each study site, a 50 µm plankton tow net was used to collect veligers and other zooplankton by either vertical tows through the water column at the lake or pumping through the net below a tap outlet at the WTP. Planktonic quagga mussel veligers range in size from approximately 57 to 300 µm (Rudstam 2009). The plankton tow collection was poured through a 500 µm strainer to remove large zooplankton. Multiple tows were combined into a large sample container and then brought back to the field research lab.

Concurrent with veliger collection, water from the study sites was collected into carboys. This water was passed through a 10 µm filter during collection. Study site water and veliger collection containers were transported back to the lab for use in testing that same day. Following testing, veligers and test water were decontaminated and disposed of in accordance with CDFW protocols, per the Scientific Collecting Permit.

Veliger toxicity testing methods

At the lab, plankton tow collections were concentrated by passing the collection through a 50 µm filter and backflushing contents into a beaker using approximately 50 to 150 mL of 10 µm filtered water collected from the study site. This 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) specimens. If veliger concentrate contained more than 5% dead-to-live veliger density, the plankton collection was not used and additional collections were made as needed. The veliger concentrate was divided into testing beakers to achieve approximately 100 to 200 live veligers in each beaker, ranging in size from D-shaped (> 50 µm) to pediveligers (< 300 µm) per replicate and an appropriate amount of 10 µm filtered site water was added to achieve a total volume of 50 mL. This division was completed within 1 hour of the density evaluation.

A stock solution of EarthTec QZ was prepared for each round of testing by diluting the chemical into 6 L of 10 µm filtered water collected from the test site. For each concentration tested, a 1.1X stock solution was prepared and the concentration was confirmed via copper measurements (free and total) prior to dosing test beakers. The target concentration for each test condition was achieved by adding 450 mL of the corresponding stock solution to individual beakers containing 50 mL of filtered water from the test site and veliger concentrate. The concentrations tested were 0, 3, 16.7, 33.4, and 50.1 µL/L of EarthTec QZ (equivalent to 0, 0.18, 1.0, 2.0, 3.0 mg/L as copper). Testing duration for each concentration was 0.5, 2, 5, and 24 h. Exposure duration started when the stock solution was added to the beaker.

Beakers were placed into a water bath to maintain a testing temperature of either 15 or 20 °C. At each testing temperature, each concentration-exposure duration combination was replicated 3 times. At the start of the experiment, temperature, dissolved oxygen, pH, and conductivity were measured with a HACH HQ40d with LDO101, CDC401, and PHC201 probes (HACH, Loveland, CO). Free and total copper and total chlorine were measured with the HACH Bicinchoninate method with the free copper reagent and hydrosulfite reagent powder pillows and HACH Total Diethyl-p-phenylene Diamine (DPD) Colorimetric Method.

At the end of the exposure duration, each test beaker was evaluated for temperature, dissolved oxygen, pH, conductivity, free and total copper, and total chlorine. At the end of the exposure duration, the beaker contents were poured through a 50 µm filter, and the beaker was rinsed three times with 10 µm filtered test site water. The 50 µm filter containing the tested veligers was placed into a dish and fast green stain was poured over the veligers (Stockton-Fiti and Claudi 2017). After staining, the veligers were rinsed into a collection cup with approximately 2 to 4 mL and then analyzed with microscopy. During microscopic examination, a 2 mL sample of veliger concentrate was removed from each collection cup 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 empty shells of veligers were open, empty shells that were not stained and had no mantle present. Counts included empty shells to assess veliger health in the samples taken from each location. Large numbers of empty shells can indicate prior treatment or unfavorable conditions at the sample location; less than 10% empty shells indicate a normal population. Approximately 100 veligers were assessed per beaker.

After analysis with microscopy, the veliger collection was returned to the testing beaker and filled with approximately 500 mL of filtered study site water. These beakers were held an additional 24 hours at testing temperature to account for the ability of the veligers to recover after an EarthTec QZ treatment. A subset of the beakers with original mortality greater than control mortality were reevaluated with the fast green stain procedure to determine mortality. The data from this recovery evaluation was considered final mortality and utilized in statistical analysis.

Each sample collection was evaluated to determine the proportion of the veliger size classes present (Table 1) i.e., D-shaped (50–150 µm), small umbonal (150–250 µm), large umbonal (200–350 µm), and pediveliger (350–500 µm).

Table 1. Size class distribution of veligers (as %) used in testing where each study site was ranked by the most abundant density of large veligers to small veligers.

Study Site	Size Class				Rank (score)
	D-shaped (50–150 µm)	Small umbonal (150–250 µm)	Large umbonal (200–350 µm)	Pediveliger (350–500 µm)	
2021 Lake Mathews (20 °C)	32%	18%	38%	11%	A (8)
Lake Piru (15 & 20 °C)	45%	15%	20%	20%	B (9)
2021 Lake Mathews (15 °C)	52%	22%	23%	3%	C (16)
2021 WTP influent (15 °C)	74%	8%	17%	2%	D (17)
2020 Lake Mathews (20 °C)	50%	30%	15%	5%	E (20)
2021 WTP influent (20 °C)	53%	25%	22%	1%	F (22)
2020 WTP influent (15 & 20 °C)	80%	15%	3%	2%	G (23)
2020 Lake Mathews (15 °C)	75%	20%	4%	1%	H (26)

Results for samples taken during the same week from a given study site were averaged. Size distributions from all the sample collections were then ranked by size class to compare the overall veliger sizes among the different sample collections. The ranking system assigned lower numerical values to a sample collection with higher proportions of larger sized veligers. The two larger size classes (i.e., pediveliger and large umbonal) were ranked from most to least abundant (1 to 8). An opposite approach was used for the smaller two size classes (i.e., small umbonal and D-shaped), where the ranking values indicated least to most abundant (1 to 8). The scores for each sample collection were summed and then ranked from A (lowest value, most abundant large veligers) to H (highest value, most abundant small veligers), revealing which samples had a higher proportion of large veligers compared to those containing small veligers (Table 1).

Non-target toxicity testing methods

The toxicity of aquatic species other than quagga mussels was assessed at each of the three study sites in parallel with the veliger dose-response testing. EPA's standard test methods (EPA-821-R-02-012, 2002 and EPA/600/4-90/027F, 1993), were used for measuring the acute toxicity of freshwater organisms over a 96-hour exposure time with analysis completed by Aquatic Bioassay and Consulting Laboratory (ABC Labs; Ventura, CA). At the suggestion of the CDFW, three non-target freshwater aquatic species were selected as indicator species used for the acute toxicity tests, including fathead minnow (*Pimephales promelas* (Rafinesque, 1820)), water flea (*Ceriodaphnia dubia* (Richard, 1894)), and rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)). The indicator species were tested in water collected from each of the three sites at the time of sample collection for the veliger dose-response testing and exposed to a dose of EarthTec QZ that was informed by the results from the veliger testing from the same study site.

Additional water quality testing

Several water quality parameters were measured in the raw water from each of these study sites. The water quality parameters were selected based on the potential impacts to biological uptake and mortality, the form of copper present, and general physical qualities that might differentiate the three study sites. The EarthTec QZ label notes that an increase in the potential acute toxicity to non-target organisms can result from its use with low pH (≤ 6.5), low dissolved organic carbon (≤ 3.0 mg/L), and low alkalinity (< 50 mg/L) water conditions. As such, the following parameters were monitored from each study site, using the specified field and/or laboratory test methods: alkalinity (HACH Colorimetric Method 10283), hardness (HACH Calmagite Colorimetric Method 8030), total and free copper (USEPA Bicinchoninate Method 8506), total chlorine (USEPA Total Diethyl-p-phenylene Diamine Method 8167), oxidation-reduction potential (ORP; Direct Measurement Method 10228), chemical oxygen demand (USEPA Reactor Digestion Method 8000), dissolved organic carbon (USEPA 415.3), pH (USEPA Electrode Method 8156), dissolved oxygen (USEPA Direct Measurement Method 10360), and temperature (digital thermometer).

Data analysis

Mortality for Lake Piru and Lake Mathews was calculated by 1 minus survival where survival was calculated as the sum of live veligers over total number of veligers observed (live, dead, and empty) for each replicate of each treatment. Empty shells accounted for about 0.6% of total count for Lake Piru and 6.4% for Lake Mathews. However, there were more empty shells in WTP influent samples, consisting of about 26% of the sample count. For WTP influent veligers, the empty shells were not included in the analysis due to the magnitude present both before and after testing, therefore mortality was calculated as dead over total number (live and dead).

Statistical analysis was completed on the veliger mortality data to look for significant trends. The recovery mortality data was used to perform linear regression modeling in R 4.1.1 (R Core Team 2021). To get the data to fit normality assumptions, the recovery mortality was transformed with a square root of the arcsine value of the recovery mortality. A general linear model (GLM) was used to evaluate the relationships between temperature, concentration, exposure time, study site, and veliger size class for mortality and each of the measured water quality parameters to determine differences with packages lme4 (Bates et al. 2015), multcomp (Hothorn et al. 2008) and car (Fox and Weisberg 2019). Tukey's test was used to determine how the variables related to each other and if significant differences were found using package multcompView (Graves et al. 2019). The package rcompanion (Mangiafico 2021) was used to compare the different models.

To further simplify the model for graphing purposes, the dosed copper concentration from an individual test condition was multiplied by the testing duration to get a time-dose variable (Table 2). Testing temperature (15 or 20 °C) and year conducted were not significant variables in mortality results, therefore results were combined for each study site.

Table 2. Time-dose variable calculation. Units are hours of exposure duration multiplied by concentration of EarthTec QZ as Cu (h*mg/L Cu)

Concentration as EarthTec QZ (as Cu)	Duration (hours)			
	0.5	2	5	24
3 µL/L (0.18 mg/L)	0.09	0.36	0.9	4.32
16.7 µL/L (1 mg/L)	0.5	2	5	24
33.4 µL/L (2 mg/L)	1	4	10	48
50.1 µL/L (3 mg/L)	1.5	6	15	72

Lethal concentration and lethal time statistics were calculated with the package ecotox (Hlina et al. 2021), eliminating empty shells from the total veliger counts. The count of dead was used as the response variable to perform probit modeling. This modeling combined the data by temperature and year conducted for each study site as these variables were not found to be significant in previous modeling statistics.

Results

Susceptibility of quagga veligers to EarthTec QZ

At all study sites, the percentage of veliger mortality increased as the concentration of EarthTec QZ increased. Percent veliger mortality also increased with exposure duration to EarthTec QZ. Mortality at the end of each exposure time was less than the mortality after the additional 24-hour recovery period for all three study sites. Statistical analysis of the recovery data showed that EarthTec QZ concentration, exposure duration, and study site were significant variables. Recovery mortality results for the veliger dose-response test conditions are presented in Figure 2.

The veligers collected at the WTP influent were the most susceptible to EarthTec QZ; they exhibited the highest mortality with shorter exposure durations at lower concentrations (Figure 2) when compared with the corresponding results from Lake Piru and Lake Mathews. Veligers from Lake Piru had the lowest mortality at each exposure duration for each concentration of EarthTec QZ tested. In general, veligers from Lake Mathews had mortality responses closer to the observed response of WTP influent veligers.

Recalling the established drinking water secondary MCL for copper of 1.0 mg/L as Cu, the results for the 16.7 µL/L EarthTec QZ dose (1.0 mg/L as Cu) test condition (see Figure 2, part A) were the main point of reference for assessing the efficacy of EarthTec QZ as a viable treatment strategy for achieving rapid (< 24 h) mortality of veligers. In the tests with WTP influent, greater than 85% mortality was achieved after 5 hours of exposure to a concentration of 16.7 µL/L EarthTec QZ at both temperatures. Mortality increased with greater exposure time and concentration. The 33.4 and 50.1 µL/L EarthTec QZ treatments with the WTP influent achieved 100% mortality after 5 hours of exposure duration. After 24 hours of exposure time, the 16.7 µL/L concentration achieved 100% mortality. Veligers from

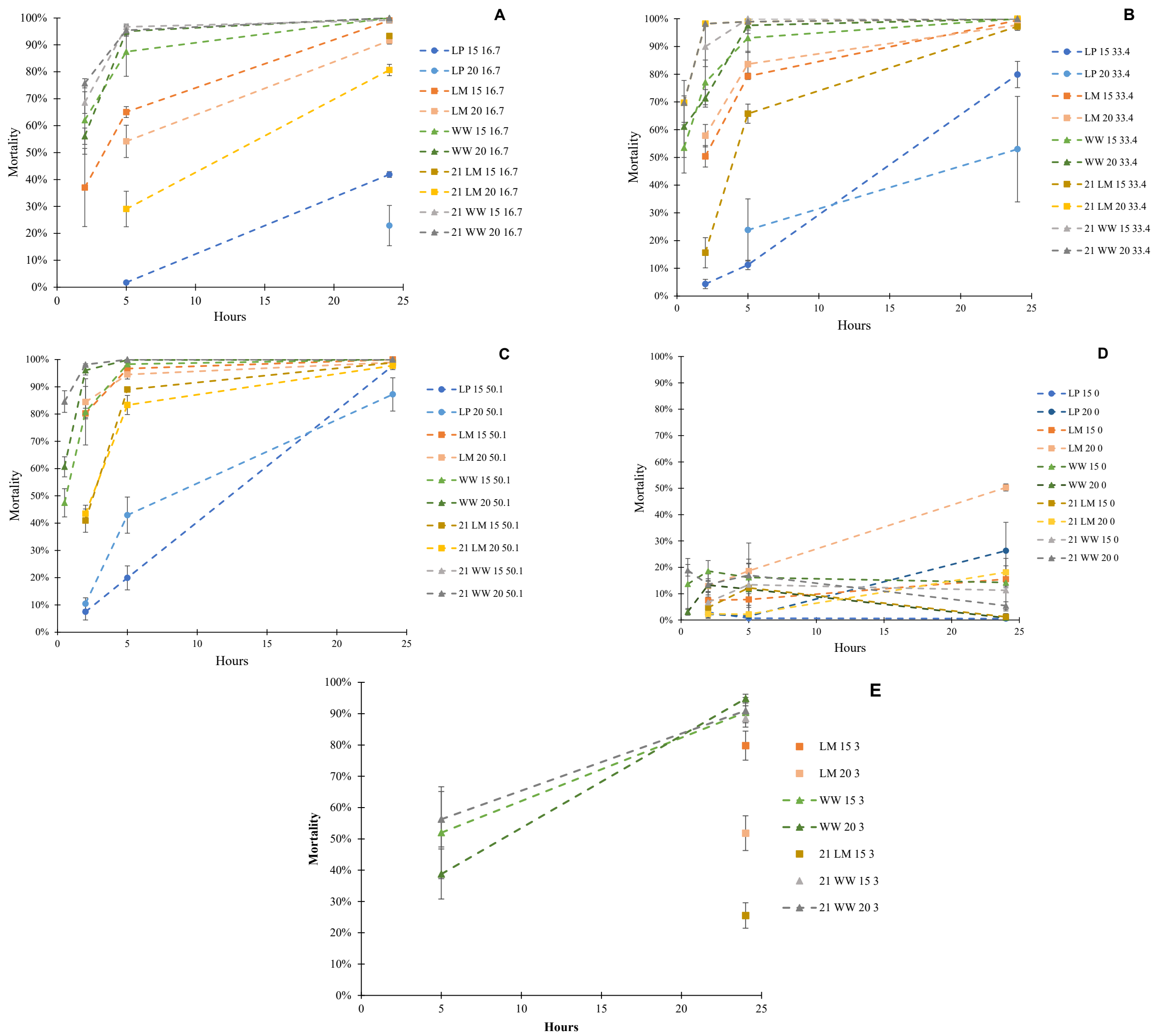

Figure 2. Veliger dose-response mortality with standard deviation bars for each test condition at the three study sites: Lake Piru (LP), Lake Mathews (LM) in 2020 and 2021 (21), and WTP influent (WW) in 2020 and 2021(21) at the two test temperatures (15 & 20 °C). (A) 16.7 µL/L EarthTec QZ (1 mg/L Cu); (B) 33.4 µL/L EarthTec QZ (2 mg/L Cu); (C) 50.1 µL/L EarthTec QZ (3 mg/L Cu); (D) 0 µL/L control mortality; (E) 3 µL/L EarthTec QZ (0.18 mg/L Cu).

Lake Mathews were not as susceptible to EarthTec QZ; an average of 60% mortality occurred after the 5-hour exposure duration at 16.7 µL/L EarthTec QZ (1.0 mg/L as Cu). After the 24-hour exposure duration in 16.7 µL/L EarthTec QZ the veliger mortality was 95%. Mortality also increased with increased concentration and exposure duration in Lake Mathews tests. Veligers tested from Lake Piru had approximately 30% mortality at the 24-hour exposure duration at 16.7 µL/L EarthTec QZ (1.0 mg/L as Cu). The two higher concentrations tested, 33.4 and 50.1 µL/L EarthTec QZ, exhibited higher mortality, but only the 50.1 µL/L EarthTec QZ achieved greater than 90% mortality after a 24-hour exposure duration (Figure 2, parts B and C).

Veliger mortality associated with the control conditions was usually less than 20%; however, there were two exceptions, both for 24-hour recovery samples (Figure 2, part D) – Lake Piru at 20 °C and Lake Mathews 2020 at 20 °C. The control showed that there was a baseline mortality, which increased with exposure duration during testing, regardless of EarthTec QZ dosing. As such, longer duration (> 48 h) dose-response studies are not expected to be representative without flowing water. The lowest dose tested was 3 μL/L EarthTec QZ. Although this condition achieved high mortality (> 85%) in the WTP influent samples at 24 hours of exposure duration, the Lake Mathews and Lake Piru samples did not exhibit significant increased mortality when compared with the control (Figure 2, part E).

All water quality parameters measured were within the suitability range for veliger survival during testing. During testing the pH decreased with increasing EarthTec QZ concentration, where the highest concentration (50 μL/L EarthTec QZ) was 0.3 units lower than the control. The pH for each experimental condition was stable during testing. With increased EarthTec QZ concentration, the free and total copper concentrations increased as expected. Copper concentrations decreased over the 24-hour exposure duration for each experimental condition (EarthTec QZ concentration and temperature) but were still within 20% of the initial dosed concentration for all study sites. Chlorine was measured at Lake Mathews and WTP study sites because water from the WTP had received a free chlorine treatment approximately 8 hours upstream, as the water enters the pipeline from Lake Mathews. Despite the upstream chlorine addition, the measured residual chlorine levels in the WTP influent samples (0.06 mg/L; Table 3) were at levels approaching the method detection limit (0.02 mg/L) and were expected to be related to interferences from other ions in the water and not residual oxidant. These measurements were consistent with levels measured from Lake Mathews samples (0.02 mg/L; Table 3), which was not expected to have any residual oxidant.

Linear regression modeling showed that the significant variables in predicting veliger mortality were concentration, exposure duration, and study site; temperature and year were not significant variables. Linear regression analysis with the time-dose and study site as variables resulted in a simplified model with a good fit (linear regression fit of $r^2 = 0.92$ (Table 4 model 1)). The lethal concentration of 50% (LC50) and 99% (LC99) mortality were calculated for each study site (Table 5). For each test location, the LC50 and LC99 decreased as exposure duration increased and corresponding confidence limits were generally progressively smaller. Lake Piru had the highest LC50 and LC99 values and the WTP study site had the lowest. The same trends were seen with the lethal time calculations (Table 6). Lethal time to 50 or 99% mortality for each test location decreased as EarthTec QZ concentration increased, and the WTP study site showed the fastest time to mortality relative to the corresponding EarthTec QZ exposures at the other sites.

Mortality results are presented by study site as a function of time-dose in Figure 3. This modeling showed that the veligers collected from the WTP influent had the highest mortality with the shortest exposure time. Veligers from Lake Mathews were still susceptible but longer exposure durations

Table 3. Raw water quality readings from each study site at the time of veliger testing.

Parameter			Total Copper	Free Copper	Total Chlorine	Oxidative Reduction Potential	Dissolved Oxygen	Conductivity	pH	Alkalinity	Hardness	Dissolved Organic Carbon	Chemical Oxygen Demand
Study Site	Year	Temperature Condition	mg/L	mg/L	mg/L	mV	mg/L	µS/cm	pH unit	mg/L as CaCO3		mg/L	mg/L
		Raw Water Quality											
Lake Piru	2019	15 °C Tests	0.15	0.14	0.08	210.4	10.45	927	8.43	187	314	5.33	10.00
		20 °C Tests	0.16	0.16	0.12	191.9	8.88	913	8.25	154	316	5.05	5.65
		Raw Water Quality											
Lake Mathews	2020	15 °C Tests	0.02	0.02	0.02	212.5	7.57	946	8.18	115	138	3.01	4.67
		20 °C Tests	0.01	0.01	0.02	245	8.08	937	8.20	115	142	2.95	5.69
	2021	15 °C Tests	0.00	0.00	0.01	185.6	8.46	950	8.24	138	281	2.98	4.65
		20 °C Tests	0.00	0.00	0.00	208.2	8.34	957	8.29	135	178	2.91	3.30
		Raw Water Quality											
WTP Influent	2020	15 °C Tests	0.01	0.01	0.04	214	8.27	944	8.02	119	150	4.09	8.50
		20 °C Tests	0.00	0.00	0.07	223.5	8.14	941	8.13	121	139	4.19	8.24
		Additional Sampling[a]	0.02	0.02	0.05	211	8.19	955	8.12	119	145	4.05	7.88
	2021	15 °C Tests	0.02	0.03	0.10	195.6	8.69	969	8.18	137	275	3.01	4.46
		20 °C Tests	0.00	0.00	0.02	218.2	8.34	961	8.28	135	186	3.12	4.86

[a]Re-sample of WTP influent for acute toxicity testing with 3 µL/L EarthTec QZ. This water was not used for veliger testing.

Table 4. Model analysis from simple to most complex to predict veliger mortality given the different study variables. Linear regression modeling results are presented to determine the best model fit.

Model # (# of variables)	Model Description
1 (3)	sqrtarcsin(Veliger mortality) = Study site *Concentration*Duration
2 (4)	sqrtarcsin(Veliger mortality) = Concentration * Duration * Alkalinity * DOC
3 (5)	sqrtarcsin(Veliger mortality) = Concentration *Duration * Alkalinity * DOC * COD
4 (4)	sqrtarcsin(Veliger mortality) = Concentration* Duration * Alkalinity * VeligerRank
5 (5)	sqrtarcsin(Veliger mortality) = Concentration* Duration * Alkalinity * DOC * VeligerRank
6 (6)	sqrtarcsin(Veliger mortality) = Concentration * Duration * Alkalinity * DOC * COD * VeligerRank
7 (6)	sqrtarcsin(Veliger mortality) = Study site* Concentration* Duration * Alkalinity * DOC * COD
8 (7)	sqrtarcsin(Veliger mortality) = Study site *Concentration * Duration * Alkalinity * DOC * COD *VeligerRank

Model #	Rank	Df. Res	AIC	AICc	BIC	R.squared	Adj. R. Sq	p.value	Shapiro.W	Shapiro.p
1	41	346	−470.8	−447.6	−229.4	0.9163	0.9066	6.46E-162	0.9849	0.00047
2	64	323	−242.5	−199	78.12	0.8638	0.8373	4.21E-107	0.9801	3.59E-05
3	112	275	−651.1	−419.2	−13.78	0.9687	0.956	1.00E-159	0.9635	3.11E-08
4	129	258	−436.7	2744	834	0.9761	0.9643	3.97E-159	0.9342	4.87E-12
5	129	258	203.3	−3024	2741	0.9761	0.9643	3.97E-159	0.9342	4.87E-12
6	129	258	1483	−2187	6554	0.9761	0.9643	3.97E-159	0.9342	4.87E-12
7	129	258	−116.7	−4998	1787	0.9761	0.9643	3.97E-159	0.9342	4.87E-12
8	129	258	6603	−1939	21810	0.9761	0.9643	3.97E-159	0.9342	4.87E-12

and higher concentrations were required to achieve close to 100% mortality (Figure 3). Similarly, veligers from Lake Piru were less susceptible and a long exposure duration was needed to obtain close to 100% mortality.

Susceptibility of non-targets to EarthTec QZ

The water flea was the most susceptible non-target organism to EarthTec QZ (Figure 4) at all study sites. Mortality results for the water flea in WTP

Table 5. Calculated lethal concentration as copper for veligers exposed for the testing durations with associated lower and upper confidence limits and standard errors for each testing site.

Duration (h)	Lethal Concentration	Dose as Cu (mg/L)	Lower Confidence Limit (mg/L)	Upper Confidence Limit (mg/L)	Standard Error
Lake Piru					
2	LC50	18.52	6.46	Not Determined	1.75
	LC99	402.03	26.68	Not Determined	4.26
5	LC50	4.05	3.29	6.41	1.04
	LC99	19.86	10.30	101.22	1.13
24	LC50	1.42	1.16	1.65	1.02
	LC99	5.97	4.20	12.19	1.05
Lake Mathews					
2	LC50	2.19	0.36	4.60	1.03
	LC99	76.94	13.63	5.53E+33	1.38
5	LC50	1.06	0.86	1.23	1.03
	LC99	7.88	5.77	13.11	1.07
24	LC50	0.17	0.13	0.22	1.05
	LC99	3.76	2.73	5.78	1.06
WTP Influent					
2	LC50	0.53	0.04	0.91	1.11
	LC99	19.24	7.22	2299.21	1.23
5	LC50	0.20	0.16	0.23	1.04
	LC99	2.24	1.76	3.06	1.06
24	LC50	0.05	0.03	0.07	1.17
	LC99	0.63	0.49	0.92	1.12

Table 6. Calculated lethal time as hours for veligers exposed to the testing concentrations of EarthTec QZ with associated lower and upper confidence limits and standard errors for each testing site..

Concentration of EarthTec QZ (µL/L)	Lethal Time	Time (h)	Lower Confidence Limit (h)	Upper Confidence Limit (h)	Standard Error
Lake Piru					
16.7	LT50	38.64	27.91	77.72	1.06
	LT99	373.10	143.21	4394.25	1.21
33.4	LT50	15.43	11.45	22.79	1.03
	LT99	236.40	106.03	1074.47	1.11
50.1	LT50	6.98	5.98	8.30	1.02
	LT99	52.33	35.86	88.92	1.07
Lake Mathews					
16.7	LT50	4.38	3.07	5.71	1.04
	LT99	116.24	62.40	334.53	1.11
33.4	LT50	2.52	2.03	3.00	1.03
	LT99	37.94	25.78	66.74	1.08
50.1	LT50	1.38	0.90	1.79	1.04
	LT99	18.97	11.93	44.61	1.08
WTP Influent					
16.7	LT50	1.45	1.04	1.77	1.05
	LT99	9.11	6.75	15.60	1.08
33.4	LT50	0.39	0.23	0.56	1.09
	LT99	11.01	7.33	20.65	1.10
50.1	LT50	0.32	0.12	0.52	1.09
	LT99	8.72	4.97	26.32	1.10

influent water were highly variable; 2020 testing yielded mortality of 0% at 8.35 and 16.7 µL/L EarthTec QZ (0.5 and 1.0 mg/L as Cu), but 100% in 3 µL/L EarthTec QZ (0.18 mg/L as Cu). Upon repetition 2 weeks later of the 3 µL/L EarthTec QZ (0.18 mg/L as Cu) concentration, the water flea had 0% mortality. In 2021, the water flea had 100% mortality at all three concentrations tested (3, 8.35 and 16.7 µL/L EarthTec QZ or 0.18, 0.5 and 1.0 mg/L as Cu). This yielded large standard deviation bars and inconclusive

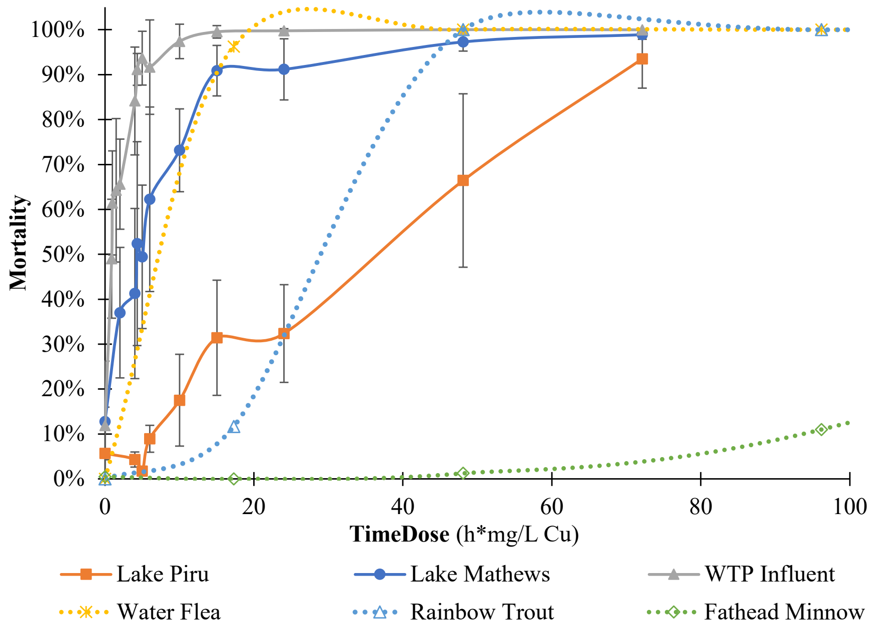

Figure 3. Mortality of veligers by study site and water flea, rainbow trout, and fathead minnow (non-target indicator results for all study sites combined) with cumulative exposure to EarthTec QZ.

results for these test conditions. A quality assurance/quality control review by ABC Labs did not identify any deviation from the test protocol, though the size class of test subjects can vary among tests. Although the difference in results is not understood, the repeated test results with 100% mortality were used in analysis to provide conservative estimates for evaluating EarthTec QZ toxicity for the non-target indicator species.

Rainbow trout were consistently sensitive to EarthTec QZ exposure, and 100% mortality was observed for all test waters with EarthTec QZ concentrations of 8.35 µL/L (0.5 mg/L as Cu) or greater (Figure 4). Fathead minnows were not as sensitive to EarthTec QZ (Figure 4). In Lake Mathews and WTP influent waters, little-to-no mortality was observed at 16.7 µL/L EarthTec QZ (1.0 mg/L as Cu). By contrast, 42.5% mortality was observed for fathead minnows in Lake Piru water at 16.7 µL/L EarthTec QZ (1.0 mg/L as Cu). However, it should be noted that Lake Piru water had an initial copper concentration of 0.15 mg/L (Table 3), whereas Lake Mathews and WTP influent waters had an initial copper concentration of 0.01 mg/L. This increased the concentration of copper in the Lake Piru test condition to a total 1.15 mg/L as Cu, which could have resulted in reaching a tipping point in the toxicity to fathead minnows.

Overall veliger mortality was highest with the WTP influent water and was achieved with lower cumulative exposure (e.g., shorter time exposure, lower EarthTec QZ dose, or both) to EarthTec QZ relative to the exposure required for similar mortality levels in the water flea, rainbow trout, or fathead minnow (Figure 3). Veligers from Lake Mathews had approximately the same mortality rate as the water flea and died with lower cumulative EarthTec QZ exposure relative to the rainbow trout and the fathead minnow.

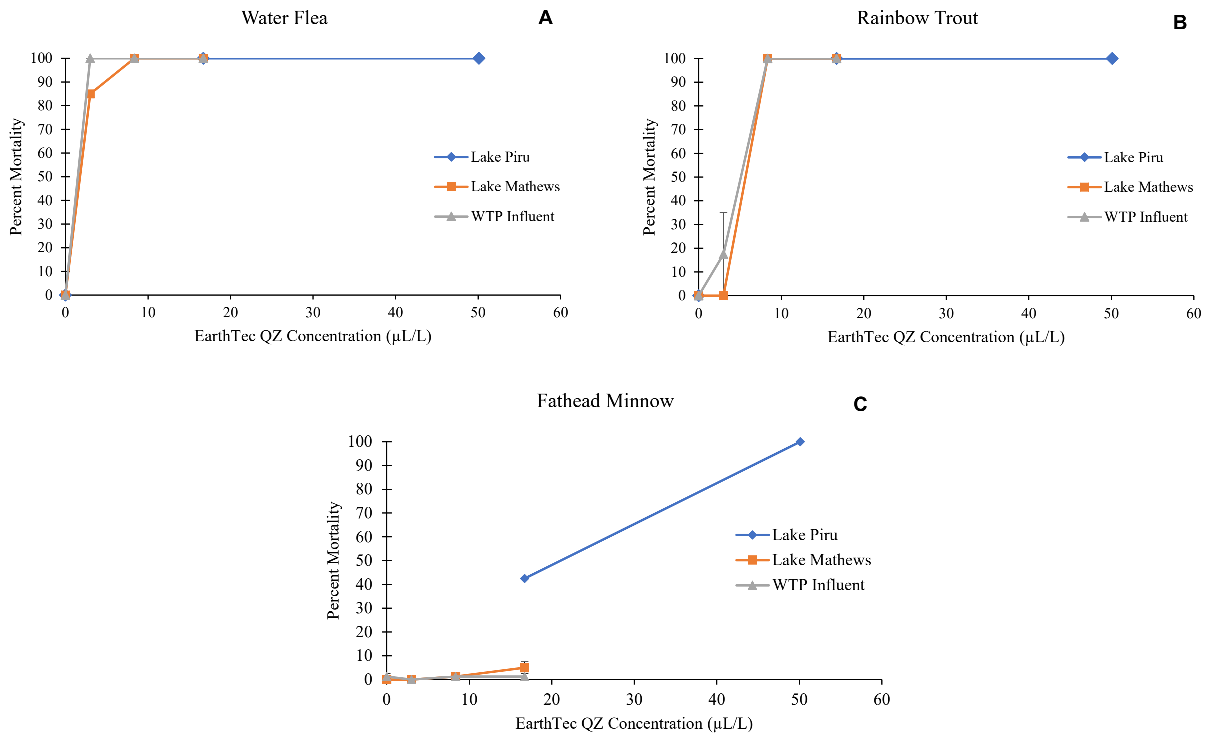

Figure 4. Average acute toxicity for (A) water flea; (B) rainbow trout; and (C) fathead minnow with EarthTec QZ following 96-hour exposure durations with standard deviation bars for each study site combining all acute toxicity results.

Veligers from Lake Piru survived higher cumulative exposure to EarthTec QZ relative to the water flea and rainbow trout (above 24 h*mg/L exposure) but had higher mortality with less cumulative EarthTec QZ exposure compared to the fathead minnow.

Raw water quality

Lake Mathews and the WTP influent had the same measured values for many of the raw water quality parameters (Table 3), which was expected since the WTP receives chlorinated water from Lake Mathews. Background copper levels (total and free) were higher at Lake Piru relative to Lake Mathews and the WTP influent. Measured residual chlorine levels were also higher in Lake Piru water, but this could have been due to interferences in testing or residual levels related to washing the test vials with tap water. The ORP levels were consistent for all samples, suggesting that there was no residual chlorine present. Dissolved oxygen, conductivity, and pH were at levels suitable for organism growth and survival.

The alkalinity of Lake Piru (mean = 171 mg/L as $CaCO_3$) was higher than Lake Mathews and the WTP influent (mean = 126 mg/L as $CaCO_3$), which indicated that Lake Piru water was able to buffer the addition of EarthTec QZ (a very acidic compound). Hardness measurements were also highest for Lake Piru (mean = 315 mg/L as $CaCO_3$). Tests conducted in June 2021 for Lake Mathews and the WTP influent water had higher hardness measurements (mean = 278 mg/L as $CaCO_3$) than samples from

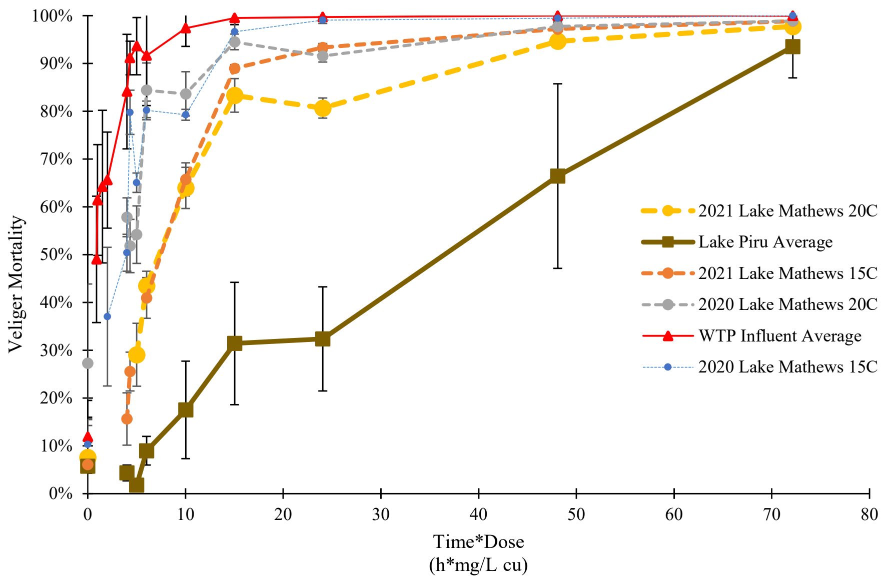

Figure 5. Average veliger mortality with standard deviation bars after exposure to EarthTec QZ ranked by abundance of large sized veligers. All temperatures and years tested for Lake Piru and WTP influent are combined. Lake Mathews veliger mortality data was not combined by temperature or sampling year to show how size class varied at the sample site. (E) 3 µL/L EarthTec QZ (0.18 mg/L Cu)

the same study sites collected 2 weeks later in July 2021 (mean = 182 mg/L as $CaCO_3$). Water from the 2020 sampling events at these study sites had the lowest hardness (mean = 143 mg/L as $CaCO_3$). The literature indicates that calcium competes with copper for binding sites in low hardness waters, but this is not a factor in waters that are considered hard like the waters from both Lake Piru and Lake Mathews (Parametrix and Hydro Qual 2006). DOC levels were different by study site, with the highest levels measured at Lake Piru (Table 3). COD varied widely, and no trend was apparent.

Water quality was a principal factor in determining the toxicity of EarthTec QZ to veligers. Lake Piru water quality was different from Lake Mathews water, especially the alkalinity and DOC measurements. When the water quality parameters were applied to the veliger logistic regression model, alkalinity, DOC, and COD were identified as significant variables, whereas hardness was not found to be a significant variable in the model.

During the 2021 sampling event, veligers from the 20 °C Lake Mathews test conditions were most abundant in the largest size classes and had a similar overall veliger size class distribution to those of Lake Piru. The veliger mortality for this sampling event was the lowest of the four Lake Mathews testing events (Figure 5). On the other hand, the smallest veligers observed during testing were from the 15 °C test conditions associated with the 2020 Lake Mathews sampling event, and the mortality was similar to the average of the results from the testing with the WTP influent, which had an abundant number of small veligers present during testing (Figure 5). This finding showed that veliger size had some influence on the mortality results.

Table 7. Veliger mortality (as %) after exposure to 16.7 µL/L (1.0 mg/L as Cu) EarthTec QZ concentration at the three study sites.

Study Sites	Exposure Time (hours)	
	5	24
WTP Influent	92.5%	100%
Lake Mathews	60%	95%
Lake Piru	5%	30%

The simplest and best fitting model showed that mortality was a function of concentration, exposure duration, alkalinity, DOC, and veliger size-class rank (Table 4, model 4). The study site variable could be replaced with alkalinity and DOC, while only minimally changing the fit of the model (Table 4, model 1 compared to model 3). Veliger size class helped explain some of the variation in the model, but this factor had a similar model fit to adding in COD (Table 4, model 4 vs. model 7).

Discussion

At all study sites, veliger mortality was achieved in less than a 24-hour exposure duration to various concentrations of EarthTec QZ. As expected, veliger mortality increased with increased exposure duration and increased concentration. The mortality was higher after a 24-hour recovery period in fresh water than the initial observed mortality post-exposure. This indicated that the toxicity of EarthTec QZ does not have immediate results, and that additional time is needed to observe the full impact of veliger toxicity to copper. The size of veliger treated also impacted the mortality results where smaller veligers died faster with less copper than the larger pediveliger life stages. Seasonality was not a significant variable in this study in determining veliger mortality; there were no significant differences in mortality when comparing the different temperatures tested or the testing with samples collected from different years for the Lake Mathews and WTP influent waters.

Since the established drinking water limit for copper is 1.0 mg/L as Cu in the US and the surface waters included in this study are also Southern California drinking water sources, we referenced our findings against this concentration. The veligers collected from the WTP influent were the most susceptible to treatments of 16.7 µL/L EarthTec QZ (1 mg/L as Cu) with average mortality of 92.5% achieved after 5 hours of exposure and 100% mortality after 24 hours of exposure time (Table 7). In water from the Lake Mathews study site, the veligers were less susceptible to the same EarthTec QZ dose, achieving an average of 60% mortality after a 5-hour exposure duration and 95% mortality after 24 hours. Only 30% mortality was achieved after a 24-hour exposure duration with the veligers from the Lake Piru study site. By testing higher concentrations of EarthTec QZ, this study ultimately showed that all veligers were susceptible to EarthTec QZ; thus, a higher concentration or a longer exposure duration may be required to achieve 100% mortality given different study sites and/or size of veliger targeted for treatment. Further testing and monitoring would be required to determine if the residual copper values resulting from the use of EarthTec QZ would be removed in subsequent water treatment.

With respect to non-target indicator organisms evaluated, the water flea was most susceptible to EarthTec QZ at all study sites, but there was some variability of response in testing with samples collected from the WTP influent. This response requires further investigation to determine if size class of organism tested was the contributing factor to the variability. The rainbow trout were extremely sensitive to EarthTec QZ with 100% mortality at 8.35 μL/L EarthTec QZ (0.5 mg/L as Cu) for all locations, while fathead minnows were not as susceptible, exhibiting 40% mortality in Lake Piru water at 16.7 μL/L EarthTec QZ (1.0 mg/L as Cu) and only 5% mortality at the same dose in Lake Mathews water.

By sampling three separate locations, we found that water quality influenced toxicity of EarthTec QZ to veligers and fish species. In samples from the Lake Piru study site, which had very hard water (> 180 mg/L as calcium carbonate (USGS 2018)), as well as high alkalinity and DOC, the veliger mortality was lower at similar concentrations compared to the samples from the Lake Mathews study site which had lower hardness, alkalinity, and DOC. Although chlorine was added in the pipeline between them, similar water quality was observed at Lake Mathews and downstream at the WTP study sites. The major difference between these locations was the presence of smaller veligers in the samples from the WTP influent in both 2020 and 2021. The veligers sampled from the WTP study site were shown to be more susceptible to EarthTec QZ at lower doses and exposure time. No clear explanation exists for why smaller veligers were observed at the WTP study site; thus, further research is needed to independently assess the impact of both size class and pre-chlorination on mortality at this study site. It is reasonable to hypothesize that exposure to chlorination followed by the 8-hour journey in the pipeline from Lake Mathews weakened the veligers and contributed to higher and faster mortalities occurring with lower doses of copper in the WTP influent water.

The difference in the acute toxicity response observed between the two different waters (Lake Piru versus Lake Mathews/WTP influent) can potentially be further explained by the differences in water quality that affect the bioavailability of metals in water as well as competitive ions. Researchers have shown that along with calcium, other competing cations such as magnesium, sodium, and hydrogen, as well as complexing ligands (DOC, OH^-, Cl^- and CO_3^{2-}) decrease the toxicity of copper as their concentrations increase in natural waters (Di Toro et al. 2001; Parametrix and HydroQual 2006; USEPA 2007). Alkalinity can affect copper by changing which complex is bioavailable (Parametrix and HydroQual 2006). Copper toxicity in fish can be predicted using the biotic ligand model which uses a wide array of water quality parameters to predict bioavailability and toxicity, including pH, DOC, ions, alkalinity, and temperature (USEPA 2016). These water quality parameters affect the chemical speciation of copper in the water and thus determine its bioavailability. For example, alkalinity, pH, hardness, and anions affect the saturation indices for copper and the percentage of total copper existing in a dissolved form. Copper toxicity has been shown to decrease when pH and hardness increase (Welsh et al. 1996). Meanwhile, other cations, especially sodium, can compete with free copper ions for binding sites in the gills of fish (Nelson et al. 1986; Welsh et al. 1996; Parametrix and HydroQual 2006; USEPA 2016). In other studies conducted by KASF Consulting, sodium levels in Lake Piru in 2019 were lower (65 mg/L) than those measured in Colorado River water (100 mg/L) in 2015 (Moffitt et al. 2016). While sodium measurements were not measured in this study, the higher sodium concentration for Lake Mathews and the WTP influent should have provided more protection from the copper toxicity. The suite of water quality parameters for predicting toxicity with the biotic ligand model was not measured in this study; however, the water quality clearly affects copper toxicity and is likely the reason for the difference in Lake Piru fathead minnow and veliger mortality.

Results of this study contrast with much of the published literature using EarthTec QZ as a treatment solution. Watters et al. (2013) used Colorado River water at Lake Mead fish hatchery and observed 100% veliger mortality dosing with concentrations of 3 and 17 µL/L (0.18 and 1 mg/L as Cu) of EarthTec QZ for 30 minutes and 6 minutes, respectively, which is much greater mortality than observed in the present study for similar doses. Lake Mead fish hatchery is about 150 miles upstream of the intake for the CRA, which terminates at Lake Mathews. Although the water from these two study sites might be expected to be similar, allowing for comparison of the test results, ≤ 10% veliger mortality was observed after 30 minutes at 3 µL/L EarthTec QZ (0.18 mg/L as Cu) in the current study (Stockton-Fiti et al. 2022). Likewise, 92% mortality was observed at 16.7 µL/L EarthTec QZ (1 mg/L as Cu) after 24 hours of exposure (Figure 2, part A) in the present study, and further, none of the tested concentrations achieved 100% mortality after 30 minutes of exposure to EarthTec QZ. The difference in results may be attributed to laboratory methodology and/or water qualities, which were not well defined or reported in Watters et al. (2013). Watters et al. (2013) conducted their study more than 10 years prior to this study and Lake Mead could have had different water quality at that time compared to our study water which should have higher hardness, alkalinity, and mitigating ions such as sodium considering the subsequent years of drought. Another study by Hurtado et al. (2021) conducted with Lake Ontario, Canada water observed 96% veliger mortality after 10 hours of dosing with 0.3 mg/L as Cu of EarthTec QZ in a pilot flow-through system. One explanation for the improved efficacy of EarthTec QZ in that study is the lower hardness and alkalinity concentrations of their test waters, which reduced its capacity to buffer the chemical compound, causing the veligers to die more quickly.

The time-dose assessment (Figure 3) allowed for a broad comparison of the impact of EarthTec QZ exposure on both quagga veligers and non-target indicators, given the differences in experimental setup, wherein the veliger exposure times were limited to 24 hours, while the non-target indicators were all tested using a 96-hour exposure time. Further testing of co-mortalities of veligers and the non-target indicators is recommended for the expected duration of treatment because past studies have reported inconsistent findings. Hammond and Ferris (2019) visually observed no significant impacts to non-target organisms during lake-scale application, and Oliver et al. (2021) identified a dosing concentration that is lethal to invasive mudsnails, yet tolerable to brown and rainbow trout at a fish hatchery. Future studies should consider using a wide range of EarthTec QZ exposures during a full-scale trial, or using field-based test methods, such as mesocosms (e.g., pilot-scale water enclosure systems adjacent to the treatment site), to better replicate expected full-scale conditions and investigate the non-target impact of a treatment.

Practitioner recommendations

The testing in this study was focused on a copper-based chemical that could be used by water managers as a more effective tool for killing quagga veligers compared with the use of low dose free chlorine, which provides minimal control. Testing results indicate that the propriety formulation Earth Tec QZ was effective against quagga veligers, exhibiting mortalities at or above 95% for two locations (Table 7). The lower mortality observed after 24 hours for the third study site, Lake Piru, can be attributed to differences in veliger size class, fewer cumulative environmental stresses on the test organisms, and/or differences in water quality.

Given these results, managers should evaluate using higher concentrations if applying EarthTec QZ at full-scale in high hardness and alkalinity applications similar to the Southern California surface waters from this study. Variation in water qualities across different sites also impacts the availability of EarthTec QZ to non-targets organisms, therefore, additional site-specific toxicity testing is needed to compare quagga mussel veliger and non-target species mortality, while optimizing dosing schemes. Mortality of veligers was shown to increase after a 24-hour recovery period and larger veligers took more time to kill than smaller quagga mussel veligers. This study showed EarthTec QZ to be toxic to water fleas and rainbow trout following the longer 96-hour exposure time associated with the acute toxicity test, but fathead minnows were impacted less by the toxicity of EarthTec QZ under the same conditions.

An example of how a recovery period would work in practice for a municipality and applied in the field follows: EarthTec QZ would be dosed through a chemical feed pump out of a chemical storage tank and applied at a water conveyance outlet for a 5-hour exposure duration. The treated water could then be mixed with dilution water (e.g., reclaimed water or surface water that is veliger- and copper-free). Veliger mortality would continue after the exposure duration even though the copper concentration is diluted. This post treatment dilution step offers additional benefits for reducing the impact of EarthTec QZ on non-target species present in natural systems and reducing the residual copper concentrations.

Water managers need to consider permitting tasks prior to conducting a treatment with EarthTec QZ, as was the case for Lake Piru, California (UWCD 2017). To consider doing a treatment in Lake Piru, federal requirements had to be met under the Clean Water Act, the Endangered Species Act, the Federal Energy Regulatory Commission, and the Forest Service (requirements vary by location). The federal regulations that may apply to all sites include the Clean Water Act and the Endangered Species Act as chemical treatments may affect non-target organisms, especially when endangered species are involved. State of California requirements include Assembly Bill (AB) 1683, which allows CDFW to quarantine infected boats, close recreational facilities and restrict lake access, and AB 2065, which put forward a state-mandated local monitoring and control program to prevent introduction of non-native mussel species. California also has requirements under the California Fish and Game Code, the California Lake and Streambed Alteration Program, the California Endangered Species Act, the California Environmental Quality Act, and the California Department of Pesticide Regulation (CDPR). Additional local regulations may apply to the target water body. The CDPR must be considered with respect to any chemical used to control quagga and zebra mussels in any California waterways (CDPR 2014). When a product is registered with CDPR, a National Pollutant Discharge Elimination System (NPDES) permit for Residual Pesticide Discharges to the Waters of the United States is required. If unregistered with CDPR, it must undergo the registration process as part of the NPDES permit process. EarthTec QZ is registered with CDPR and EPA under its pesticide registration program (USEPA 2013) and is also NSF 60 certified.

Conclusions

The results presented in this paper lay the foundation for testing that can be conducted to assess full-scale treatment efficacy of EarthTec QZ in high hardness and alkalinity surface waters found in Southern California or other locations. Recommended next steps include conducting pilot-scale testing adjacent to treatment site (flow through testing or mesocosms), investigating modified non-target species toxicity testing with different species or size classes, and taking a closer look at permitting requirements. Future work should look at the full suite of organisms present in the treated water for realistic dosing concentrations as increased amounts of algae, DOC, etc. impact the availability of copper. Other considerations to investigate after conducting this study include understanding the synergistic effects of a chlorine treatment with low levels of EarthTec QZ and assessing additional technologies aimed at removing larger veligers (e.g., filters) upstream of an EarthTec QZ treatment.

This study indicated that low doses of EarthTec QZ may work as well as high doses but that longer exposure times are needed to obtain the same mortality. Field results and current practice using EarthTec QZ in the Midwestern United States show that low doses of 0.5 mg/L as Cu are effective at reducing and even eradicating adult quagga mussels, but the exposure duration is weeks to a month (Hammond and Ferris 2019). Specific evaluation of EarthTec QZ doses is recommended for high hardness and alkalinity surface water applications, with correlation of quagga mortality to water quality.

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Authors' contribution

K.S. was the project biologist and study lead for research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, and writing of the original manuscript.

E.O. led the water quality assessment, experimental test plan development, and permitting portions of the study and contributed to research conceptualization, sample design and methodology, data analysis and interpretation, and writing of the original manuscript.

C.P. contributed to the research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, funding provision, and writing of the original manuscript.

D.H. was a reviewer for the research conceptualization, sample design and methodology, data analysis and interpretation, and contributed to the writing of the original manuscript.

Ethics and permits

No ethics approvals were required for this study. The project team obtained a California Department of Fish and Wildlife Specific Use Scientific Collecting Permit (S-191710001-19172-001 and amendment S-191710001-19172-001-01) for the collection, transport, and sacrifice of quagga mussel veligers, as well as incidental non-target aquatic species.

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