Research Article

Challenges of eradication efforts for zebra mussels in Highline Lake, Colorado, using EarthTec® QZ

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Abstract

Significant ecological and economic threats are posed by zebra mussels, *Dreissena polymorpha*, a highly invasive aquatic species, to freshwater ecosystems. Following the detection of an isolated population of zebra mussels in Highline Lake, Colorado, in 2022, an eradication effort was initiated in spring 2023. The reservoir was lowered by approximately 9.1 meters and EarthTec® QZ, a copper-based EPA-registered molluscicide, was applied at 4 ppm (240 µg L^{-1} copper). Water samples were collected prior to, during, and following the treatment to monitor copper concentrations and water quality parameters. An average 148.95 ± 43.46 µg L^{-1} dissolved copper concentrations was observed across all treatments, with variations influenced by depth and timing of application. Sufficient copper distribution was achieved throughout the reservoir, as confirmed by water sample analysis and geostatistical modeling. However, suboptimal water temperatures (below 7 °C for extended periods) were maintained during the treatment. The continued presence of veligers and adult mussels in late summer 2023 was revealed by post-treatment monitoring, indicating an unsuccessful eradication attempt. A subsequent, more intensive retreatment in fall 2023 was completed and involved higher EarthTec QZ concentrations (8 ppm; 480 µg L^{-1} copper) prior to complete draining of the reservoir. Average copper concentrations were 384.79 ± 38.62 µg L^{-1} and slightly higher temperatures than the first treatment were observed (8.87 ± 0.98 °C). The second treatment demonstrated more effectiveness, as no living zebra mussel or veliger detections were observed in the reservoir in 2024. The reservoir was then drained to allow for complete freezing and desiccation of any remaining mussels over the winter 2024. While no live zebra mussels were detected within the reservoir post-draining, two veliger detections were recently confirmed following the refilling of Highline Lake in 2025, with reinfestation likely occurring via the Colorado River during this process. The challenges of zebra mussel eradication, particularly the importance of optimizing treatment timing with respect to water temperature, copper bioavailability, and site location are highlighted in this study.

Key words: copper-sulfate; AIS, ANS, invasive species, molluscicide, *Dreissena polymorpha*, dreissenid

Introduction

Significant ecological risks are posed by invasive species, such as zebra mussels, *Dreissena polymorpha* (Pallas, 1771), including displacement of native species, disruptions of food webs, alterations of ecosystem processes, changes in water chemistry, and damage to infrastructure (Haag et al. 1993; Strayer 1999; Zhu et al. 2006). The establishment and spread of zebra mussels are strongly associated with human activities, particularly boating. Adult mussels, when attached to watercraft, and their microscopic larvae (i.e. veligers) can be accumulated in internal compartments, including live wells, ballasts, and bilges. This allows the mussels to be transported between water bodies if watercraft are not properly drained (Frischer et al. 2009). Once transported, zebra mussels often proliferate rapidly due to limited competition and predation (Casagrandi et al. 2007). Conditions characterized by water temperatures below 30 °C, pH levels above 7.4, calcium concentrations greater than 12 mg L^{-1}, and salinity below 3 ppt support the survival of zebra mussels (Morton 1971; Spring and Rose 1988; Kovalak 1989; Sprung 1995; Wimbush et al. 2009). Consequently, the freshwater range expansion of zebra mussels has been largely dictated by their biological and environmental requirements, which are relatively non-restrictive (Vanderbush et al. 2021).

In North America, zebra mussels face little competition from species or predators, facilitating their rapid proliferation (Casagrandi et al. 2007). Thus, infestation is primarily dictated by transportation to new bodies of water, water quality, and high reproductive success of the organisms. Watercraft inspection, decontamination programs, and public education are commonly employed to prevent and control the spread of zebra mussels. For example, a "Clean, Drain, Dry" policy for all watercraft leaving a body of water is enforced by Colorado Parks and Wildlife (CPW). Despite these efforts, a reproducing population of nine adult zebra mussels in Highline Lake (Loma, Colorado) was detected by CPW in 2022. An early stage of infestation was indicated by the detections, and an eradication attempt using both physical methods and chemical treatments was completed.

EarthTec® QZ is a molluscicide designed to target dreissenid mussels. The active ingredient is a formulation of copper sulfate pentahydrate combined with an acid, and is delivered in the cupric ion form to make it more biologically available to the target organism and minimize precipitation from the water column (Hammond and Ferris 2019). Approximately 5% copper (19.8% copper pentahydrate) in liquid form with a low pH is contained in the product, which enhances the stability and bioavailability of copper in the aquatic environment (Park et al. 2015). Although the efficacy of EarthTec QZ on zebra and quagga mussels *D. bugensis* (Andrusov, 1897) has been demonstrated by laboratory and small-scale field studies, its effectiveness in larger bodies of water remains largely untested (Watters et al. 2013; Iwanyckyj et al. 2017; Carmosini et al. 2018; Hammond and Ferris 2019).

Approximately 33 attempts at dreissenid mussel control have been attempted in North America, with EarthTec QZ being utilized in 14 of these projects. Of these 14, sustained eradication, defined as the absence of mussels during long-term monitoring within the treated areas, was achieved in three instances (Hammond and Ferris 2019; Dahlberg et al. 2024). A significant limitation observed in most of the eradication efforts was the restricted success of projects wherein only a single life stage (i.e., veligers or adults) was targeted. More effective strategies consistently involved comprehensive pre-treatment assessments that accounted for detection uncertainty, and included methods such as veliger sampling via plankton tows, SCUBA diving, and thorough shoreline and underwater structure inspections for mussel presence. Most importantly, successful projects applied EarthTec QZ in concentrations designed to address all likely life stages that were present in the water body. The first successful eradication attempt occurred in Billmeyer Quarry in Pennsylvania, USA which had been infested with an established and reproducing quagga mussel population for 12 years (Hammond and Ferris 2019). Following an EarthTec QZ treatment that covered 50% of the lake, neither live veligers or adult mussels were detected through conventional or eDNA sampling for four consecutive years. Failures in eradication efforts, evidenced by the re-emergence of mussels in long-term monitoring areas, are likely attributable to the proportion of the infested area that was treated. Among the 14 projects that used EarthTec QZ, the three successful long-term eradications were all achieved in lakes where 50% of the lake area was treated, whereas significantly small proportions, averaging only 7.6% ± 15.1 of the lake area, were treated in other projects. Therefore, comprehensive pre-treatment assessments, the application of EarthTec QZ at concentrations targeting all life stages, and treatment of approximately 50% of the infested water body appear to be the most effective way to treat and eradicate dreissenid mussels.

Highline Lake, a 153 acre reservoir fed by surface-water, with a max depth of 19 meters is located at 1,430 meters in elevation in the Colorado River Watershed. The reservoir is connected to the Government Highline Canal used for agriculture irrigation in the summer months (April–October) and is drained into a small stream, Mack Wash, then to Salt Creek, prior to the Colorado River located 9.66 km downstream. The substrate is primarily silt, supports aquatic plant growth, and is a managed fishery including common carp *Cyprinus carpio* (Linnaeus, 1758), gizzard shad *Dorosoma cepedianum* (Lesueur, 1818), green sunfish *L. cyanellus* (Rafinesque, 1819), and white sucker *Catostomus commersonii* (Lacepėde, 1802) and is seasonally stocked with black crappie *Pomoxis nigromaculatus* (Lesueur, 1829), bluegill *Lepomis macrochirus* (Rafinesque, 1819), channel catfish *Ictalurus puntatus* (Rafinesque, 1818), largemouth bass *Micropterus salmoides* (Lacepėde, 1802), and hatchery-raised rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). The reservoir is a popular recreational site, and approximately 2,800 boats are accommodated per season (March–September; CPW Internal Reports) for both fishing and water sports. Prior to 2022, extensive annual zebra mussel monitoring surveys were performed such as watercraft inspections, the deployment of mussel substrates to promote attachment if present, shoreline surveys, and plankton tows for veliger collection were all completed approximately once per month from May–October; with no evidence of zebra mussels being detected in Highline Lake.

A critical gap in the literature is highlighted by the scarcity of published reports on invasive species eradication efforts, especially those detailing both success and failure (Wittenberg and Cock 2009). The development of evidence-based management strategies is hindered by the lack of published studies, despite the increasing documentation of zebra mussel detections across the United States. Thus, the successes and failures of our eradication attempt are evaluated in this study, following a comprehensive effort involving two treatments with EarthTec QZ. Throughout this process, water quality analysis results, a Biotic Ligand Model, and a geostatistical modeling technique were utilized to estimate copper toxicity and distribution, and sentinel cages containing Asian clams *Corbicula* sp. were deployed to monitor the effects of the treatments against zebra mussels. Our efforts were strategically timed to coincide with the seasonal closure of the Government Highline Canal (November–April), which feeds the reservoir during the agricultural irrigation season (April–October). With this limited operational window, the completion of this critical eradication attempt was prioritized by our management team during the hydrologic closure. After the second treatment, the reservoir was drained to facilitate the complete freezing and desiccation of any remaining mussels over the winter of 2024. The second treatment and draining were considered promising and likely resulted in the eradication of mussels from Highline Lake. However, while no live zebra mussels were detected within the reservoir post-draining, two veliger detections were recently confirmed following the refilling of Highline Lake in 2025, with reintroduction likely occurring from the Colorado River via the Government Highline Canal during this process.

Materials and methods

Upon the detection of nine adult zebra mussels in Highline Lake in fall 2022, an extensive sampling effort was initiated by CPW to determine the upstream and downstream extent of mussel presence. In November 2022, plankton tow samples were collected by CPW from seven sites upstream of the reservoir, three sites within Highline Lake, and three sites downstream. In addition, extensive physical surveys were performed by CPW, covering 61.2 km of the Government Highline Canal and documenting 194 survey points. These surveys were focused on infrastructure and areas with slow water flow (e.g., near gates and grates) where mussels were most likely to reside. Shoreline surveys were also conducted on the Colorado River, both upstream and downstream of the Government Highline Canal diversion. All plankton tow samples were preserved with Tris buffer and 90% ethanol, with 70% of the final sample volume as ethanol, and sent to the Bureau of Reclamation (Reclamation) Technical Service Center for analysis of mussel veligers using both microscopy and polymerase chain reaction (PCR) techniques. No mussel veligers or eDNA were detected in any of the plankton tow samples. This comprehensive effort suggested the adult mussel detection was contained within Highline Lake, and treatment of only that location was prompted using EarthTec QZ. Given the absence of prior detections in Highline Lake and urgency of this project, the presence of both adult and veliger life stages was assumed. Therefore, to maximize the probability of eradication success, the maximum permissible area (50% of the lake) was treated with EarthTec QZ at concentrations targeted at both life stages, as described below. Decision-making was guided by the EarthTec QZ product label for all treatments. All methods not explicitly cited with Earth Science Laboratories, Inc. (2022) were developed by CPW consultation with the manufacturer.

Bathymetry

Prior to the application of EarthTec QZ, Highline Lake was drawn down to approximately 25% capacity, and corresponding to about 9.1 meters below its maximum level (Figure 1A). Two main purposes were served by the drawdown. First, mussels were exposed to winter freeze-thaw cycles in order to improve eradication efforts by inducing initial mussel mortality. Second, the volume of water requiring treatment with EarthTec QZ was reduced to use less product to achieve desired concentrations. After the reservoir was lowered, but prior to treatment, bathymetry data points were collected to estimate the volume of water to determine the correct EarthTec QZ dosage. Bathymetry points were collected via a raft equipped with a Lowrance sonar unit and a flat bottom boat with a Garmin Echomap Ultra High Definition Series unit. The reservoir had partial ice cover during the mapping efforts, so depths near the dam were gathered by putting the transducer through holes drilled through the ice. Depths were collected randomly across the reservoir, and GPS points were documented. The shoreline points were collected from the front of a beached raft when the shoreline was too muddy to walk and by foot in dry areas. Once the survey was completed, the data were used for the creation of an initial bathymetry map in ArcPro software.

Safety precautions

Prior to applying the EarthTec QZ, all personnel were briefed on project safety and first aid measures in the event of exposure to the eyes, skin, or ingestion of the chemical (Earth Science Laboratories Inc. 2022). All personnel were provided appropriate personal protective equipment (PPE) including waders, a Tyvek suit, face shield or goggles, an N-95 mask, and chemical-resistant gloves (Earth Science Laboratories Inc. 2022). Crews were also provided with a medical treatment plan, contact information for emergency professionals in the local area, detailed instructions for the

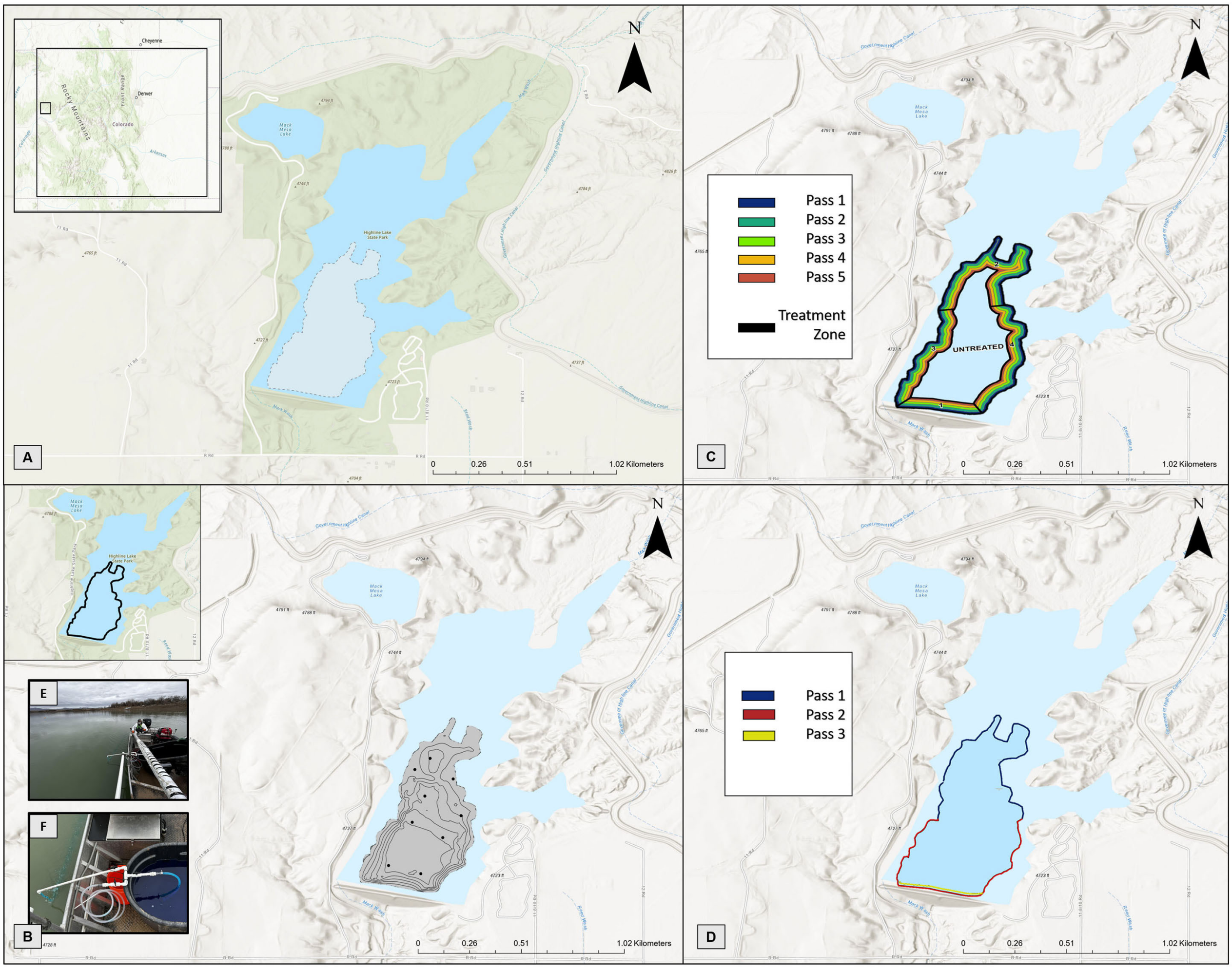

Figure 1. (A) Highline Lake located in Loma, Colorado, USA at full capacity (blue polygon outline) and after draining the reservoir to 25% capacity prior to the copper-sulfate application (dotted line, light grey polygon). (B) Contour lines represent the reservoir depths and black points represent the water sampling locations. (C) Treatment zones (black cross section lines) and pass lines for each of the four application boats during the first and second applications. Pass 1 applied the copper at 0–10 meters from shore (blue), pass 2 applied 10–20 meters from shore (dark green), pass 3 was 20–30 meters from shore (light green), pass 4 was 30–40 meters from shore (orange), and pass 5 was 40–50 meters from shore (red). Copper was administered by boat (E) at a max speed of 2.32 km h^{-1}, and applied with a submersible pump attached to a PVC manifold (F). (D) Treatment passes for each of the two application boats for the third application. Each pass was 0–10 meters from shore (blue and red), and pass 3 was an additional pass along the dam (yellow).

chemical application, and a map of intended application zones and methodologies for shore and boat applications. Each boat was supplied with a first aid kit, two one-liter water bottles for emergency eyewash, and a two-way radio. The application management team also noted that EarthTec QZ is highly corrosive to metals due to low pH (0.2), which was an additional concern because the application boats were made of aluminum. Thus, in the event of a spill on the boat, and to prevent corrosion, the crews were instructed to quickly rinse the spilled area with reservoir water (Earth Science Laboratories Inc. 2022).

Application methods

Product label guidelines for EarthTec QZ stipulate that applications be at least 14-days apart and that no more than half of the water body be treated.

This limitation is advised to minimize oxygen loss from decomposition of dead aquatic plant biomass, a process that can cause unintended fish suffocation (Earth Science Laboratories Inc. 2022). Furthermore, the label states that a concentration of 16.7 ppm of EarthTec QZ in the treated water must not be exceeded.

Acute toxicity to non-target fish species is increased by certain water quality parameters including, low pH (< 6.5), low dissolved organic carbon (DOC) levels (3.0 mg L^{-1} or lower) and soft waters (alkalinity < 50 mg L^{-1}; Earth Science Laboratories Inc. 2022). We aimed for a target concentration during the treatment of 4 ppm (240 µg L^{-1} copper) based on a preliminary study (Supplementary material Figure S1), previous knowledge of low DOC and high pH, and the volume of water in the reservoir (533.9 acre feet). It should be noted that a lower concentration for treating Highline Lake was recommended by the manufacturer. However, to increase confidence in the applications, a 4 ppm treatment was elected because this concentration was tolerated by most fish species during a bioassay. The applications were performed over three dates: February 28, March 15, and March 30, 2023.

For the first and second applications, watercraft use included four motorized boats and a raft to ensure a dispersed application of EarthTec QZ. Water samples that were collected after the first and second applications confirmed that the product dispersed evenly and could be adequately applied with fewer watercraft. To reduce logistical constraints, two motorized boats were used for the third application. Additionally, application crews were dispersed throughout the perimeters of the lake after the shore had dried enough to use backpack sprayers to apply EarthTec QZ to any exposed structures such as fish habitats, buoys, floating wave-breakers, docks, and any stagnant pools of water. All applications were made to the surface of the water, and no physical mixing of the product was performed other than the propellers of the boat as EarthTec QZ was applied. EarthTec QZ exhibits rapid dispersion throughout the treated water body, negating the need for any additional mixing (Earth Science Laboratories Inc. n.d.).

Each application boat was fit with a 378.5 L plastic trough to hold the chemical, a small Honda EU-2000 generator, and a submersible pump attached to a PVC manifold to dispense the chemical (Figure 1E, F). Troughs were filled with 132.5 L of lake water and 132.5 L of EarthTec QZ prior to the application. The chemical was diluted to reduce the likelihood of injury to crews and the severity of corrosion to the boat if a spill occurred. Corrosion to the outside of the boat was not a concern because the chemical was diluted enough once applied to the lake water. Once the troughs were filled with the 50/50 mixture, large wooden stir sticks were used to properly mix the solution. Submersible pumps were adjusted to an application rate of approximately 17.6 L min^{-1} for the product to be applied for approximately 15 minutes, allowing time for volume and spatial application to be monitored across each treatment zone (Figure 1C, D). After each pass, the boats returned to the boat ramp to refill each trough using the methods described above. Five passes were completed by each boat throughout a treatment zone for the first and second application of the product, and three passes were completed for the third.

First and second application

Since the product label allows for only 50% of the body of water to be treated at each application, EarthTec QZ was applied to the outer margins of the lake only during the first application (February 28) and the second application (March 15; Earth Science Laboratories Inc. 2022). Applications were initiated at the shoreline and proceeded outward in multiple passes, allowing fish to move to untreated areas (Earth Science Laboratories Inc. 2022). Boats were directed to dispense EarthTec QZ traveling at 2.32 km h^{-1} per pass and at intervals of 0–10 meters from shore (pass 1), 10–20 meters from shore (pass 2), 20–30 meters from shore (pass 3), 30–40 meters from shore (pass 4), and 40–50 meters from shore (pass 5; Figure 1C). Four application boats and a raft were used to apply 2,649.8 L of EarthTec QZ around the outer margins of the lake. This amount, which was determined based on the volume of water remaining in the lake (533.9-acre feet) and preliminary studies (Figure S1), was aimed to achieve a target 4 ppm concentration, 240 µg L^{-1} copper. EarthTec QZ was applied by application boat #1 in treatment zone 1 along the dam between the southwest corner and southeast corner of the dam. The product was applied by application boat #2 in treatment zone 2, along the northern shoreline. Shallow mudflats and northern coves were treated from a raft. Two passes with 113.6 L of product on the raft were completed to treat the coves and mudflats on the northern end of the reservoir. The product was applied by application boat #3 in treatment zone 3 along the western shoreline between the west side of the reservoir and southwest corner of the dam. Finally, the product was applied by application boat #4 along the eastern shoreline between the southeast corners of the dam to the east side of the reservoir located just north of the inlet channel.

Third application

Two application boats were used, to apply 757 L of product to ensure the target EarthTec QZ concentration was maintained at 4 ppm (240 µg L^{-1} copper) during a third application on March 30th. Three passes were completed by each boat along the perimeter of the reservoir 0–10 meters from shore. A measured 132.5 L of product, diluted with 132.5 L of reservoir water, was mixed into two separate stock tanks on each boat. An additional 132.5 L of product was then diluted with 132.5 L of reservoir water for application with a third pass along the dam. EarthTec QZ was applied by both application boats on the north end of the reservoir, the south end of the reservoir, and finally across the dam for a total of three passes (Figure 1D).

Water sampling

Nine sampling points were selected across the reservoir based on location, depth and accessibility via boat or shoreline (Figure 1B). These locations were strategically chosen to ensure two sampling points at each depth across the reservoir. Water quality samples were collected daily during each application, with collection starting on March 1st and continuing until the reservoir began to fill on April 11th 2023. Various types of water samples were collected at each sampling point and at various times and included dissolved copper concentrations used for analysis of treatment effects, water hardness (from total magnesium and calcium concentrations), water temperature (°C), pH, DOC, and dissolved oxygen (DO; mg L^{-1}) and other metal concentrations including aluminum, arsenic, calcium, cadmium, iron, potassium, magnesium, manganese, sodium, lead, selenium, and zinc.

Water quality parameters (pH, DO, temperature) were collected from a boat every day throughout the treatment at the surface of the water and 3 m above the bottom with a YSI ProDDS SONDE (pH: n = 615, DO n = 613, temperature n = 615) and 16 days after the reservoir began to fill (pH: n = 296, DO n = 295, temperature n = 296). Three pre-treatment water samples were collected to establish a baseline for pH, DO, and temperature.

Given that copper toxicity to mussels can be influenced by dissolved organic carbon (DOC) levels through binding with metal ions and reducing their bioavailability (Playle et al. 1993), three water samples were collected for analysis prior to the treatment for use in a Biotic Ligand Model (BLM). All samples were submitted for DOC analysis to the Colorado Department of Public Health and Environment (CDPHE), and the baseline DOC of Highline Lake was reported as 2.61 mg L^{-1}, with low DOC indicated, and was used in the BLM described below (Windward Environmental, LLC 2017).

Water samples for metal analysis were collected at each sampling location, both at the surface and 1 meter above the reservoir bottom in locations deeper than 3 m. Each sample was split into two samples, one for total (non-filtered) and one for dissolved (filtered) metal analysis. Field duplicates were collected at each site. Water samples for metal analysis were collected every 1–3 days following the initial treatment during the application period. Specifically, samples were collected on seven separate days during the first application (195 non-filtered and 199 filtered samples), five days during the second application (124 non-filtered and 146 filtered samples), and two days during the third application (5 non-filtered and 92 filtered samples). After collection, samples were sent to CPW's analytical laboratory for further processing.

Non-filtered water samples were collected by filling two 15 mL Falcon tubes with sample water from a pre-rinsed 50 mL syringe. Filtered water samples were collected from the same sample water in the syringe used for the non-filtered sample. A 30 mm, 0.45 µm polyethersulfone (PES) filter was attached to the bottom of the syringe and seeded with a few drops of the sample. The remaining sample was filtered and placed into two, 15 mL conical tubes. All samples were preserved with three drops of ultra-pure nitric acid (Ultrex® II) in 15 mL sample tubes to reach a pH less than 2 and shipped to the CPW River Watch and Toxicology Laboratory (Fort Collins, CO).

Water quality analysis

Metal concentrations were analyzed using a ThermoScientific iCAP 6000 Series Inductively Coupled Plasma, Optical Emission Spectrometer (ICP-OES) introduced using a CETAC ultrasonic nebulizer (U5000AT+) and operated with iTeva software (version 2.8.0.97). Temperature set points used in the nebulizer were 3 °C and 140 °C. Samples were injected into the plasma using a quartz torch and a 2.0 mm quartz center tube. Prior to analysis, nitrogen gas (N_2) was used to purge the instrument for a minimum of five hours before the high purity argon plasma was ignited. Plasma was allowed to stabilize for one hour before initial calibration. The instrument was calibrated using a minimum of a five-point curve to bracket the concentrations of the analytes (Table S1). Matrix solutions for blanks and standard dilutions were made in 1% HNO_3 and 1% HCl using deionized water by volume (DI; Barnstead Nanopure system). Calibration standards were composed of separate externally purchased NIST-Traceable certified standards (SRM 3100 Series). Yttrium was used as an internal standard and was introduced inline and monitored at three wavelengths (Table S1). Method detection and reporting limits were previously determined following Environmental Protection Agency (EPA) procedures (EPA Method 200.15).

After calibration, a series of initial quality control (QC) samples were analyzed including an internal calibration verification using an NIST-certified externally purchased standard (Ultra High Purity Standards), a continuing calibration verification (CCV), and a continuing calibration blank (CCB). The CCV and CCB samples were analyzed every tenth sample for the duration of the analyses. Analysis proceeded with each element if it was calibrated with a correlation coefficient value of 0.995 or greater and all subsequent QC samples were within 10% of the expected value.

Overall, the analysis lasted 220 seconds, followed by an 80-second rinse using 1% HNO_3, 1% HCl in Nanopure water by volume with three repetitions of each sample before rinsing. Duplicate samples were run every ten samples, alternating between sample duplicates and spiked duplicates. An acceptable range for duplicate samples was established at ± 10%. The first spiked duplicate consisted of an external spike and subsequent duplicate spike samples consisted of an internal standard in each analytical batch of samples. Spike recovery was considered acceptable between 85–115%. If any of the analytes fell outside of the calibration range, the sample was diluted using 1% HNO_3 and 1% HCl solution. Blanks were flagged if they were greater than 5% of the detection limit as suggested by the manufacturer or the detection limit calculated from previous sample batches. Any sample(s) that were not passed by QC tolerances, or were not bracketed by passing QC CCV/CCB samples, were reanalyzed. Water hardness was calculated from total calcium and magnesium values for each water sample.

Sentinel cages

To reduce the risk of more zebra mussel introductions into the reservoir, Asian clams were used as a sentinel species since they are suggested to be as sensitive to copper toxicity as zebra mussels (Goulder and Wong 2024). One sentinel cage was placed at each sampling location throughout Highline Lake. Cages were fabricated from 10.2 × 5.1-cm PVC pipes with mesh covering the ends and attached with zip ties. Small holes were made on the sides of the PVC to allow for water flow. At one end of the cage, the pipe was attached to a buoy, and at the other end, it was attached to a weight to keep the cage submerged. Asian clams were collected prior to the treatment from Mack Wash downstream of the reservoir dam, and five clams were placed in each of the sentinel cages. Two sentinel cages were placed below the dam in Mack Wash as a control since no treated water was being released during the applications. Cages were checked every day in the early afternoon for any mortalities.

Statistical analysis

An analysis of variance (ANOVA) was used to determine if there were detectable differences in temperature, DO, pH, or dissolved copper over the three application periods. If a difference was detected, a Tukey's Honest significance test was conducted. Significance was set at 0.05 ($\alpha = 0.05$), and all analyses were conducted in R version 4.2.2.

A geostatistical model was developed to interpolate dissolved copper concentrations throughout Highline Lake after the three EarthTec QZ applications. Geostatistical methodologies that used spatial coordinates to predict dissolved copper concentrations were applied by this model, and the surrounding measured values were weighted to derive a predicted value for the copper concentration at unmeasured locations. Specifically, average dissolved copper concentration values from the ICP-OES results at all sampling locations for the three applications were used to create a Kernel Density Model with the ArcPro Geospatial tool, to estimate copper concentrations across the reservoir as a function of the average weekly water temperature, pH, depth, and water hardness.

The Biotic Ligand Model (Windward Environmental, LLC 2017) was used to calculate site-specific water quality criteria based on water chemistry data. The BLM is based on the assumption that copper toxicity is primarily related to the amount of metal bound to a biochemical receptor on the aquatic organism. Other ligands in the water may also be bound to copper, with its accumulation at the biotic ligand and, ultimately, its toxicity being reduced. By incorporating our water quality parameters in the BLM, the final acute value (FAV) for copper toxicity at Highline Lake was estimated. To predict the FAV, the simplified site chemistry version of the BLM was used. Parameters during the treatment that were incorporated into the model included average temperature, pH, pre-treatment dissolved copper concentrations of 2.05 μg L^{-1}, pre-treatment DOC estimates of 2.61 mg L^{-1}, and water hardness (356.0 ± 34.1 mg L^{-1} $CaCO_3$). To further refine the BLM, default regional median ion ratios were included, based on delineations from the CEC (Commission for Environmental Cooperation 1997). These ratios, which included Ca:Mg 1.46, Ca:Na 0.51, Ca:K 19.04 SO_4:Cl 11.12, and pCO_2 3.2, were used to estimate the FAV for copper toxicity at Highline Lake.

Second treatment

After the initial treatment of EarthTec QZ in March 2023, veligers were detected during routine monitoring in September 2023, indicating that the primary eradication effort had not been successful. In response, an additional 21 plankton tow samples were collected by CPW from locations upstream of Highline Lake, 11 samples within the Government Highline Canal, and three samples at a power plant situated upstream of the reservoir. Negative results for zebra mussel veliger presence were confirmed by the Reclamation, but over 100 adult mussels were identified in Highline Lake in the fall of 2023. Consequently, a second, more intensive treatment was implemented at Highline Lake in November 2023, which included both a retreatment with EarthTec QZ and a planned reservoir draining. During the second treatment, approximately 2,500-acre feet of water was held in the reservoir, a substantial increase compared to the 533.9-acre feet during the first treatment. Due to the lack of mortality during the first 4 ppm treatment; the concentration of EarthTec QZ was doubled to 8 ppm (480 μg L^{-1} copper). This increased concentration aimed to eliminate the zebra mussels or, at a minimum, reduce the potential for mussel introduction before the reservoir was drained. Following this treatment, the reservoir was fully drained to encourage desiccation and freezing of any remaining mussels, representing a more aggressive management strategy. While water quality samples were collected and sentinel cages were deployed during this second treatment, the methodologies employed for data collection and analysis were not as scientifically rigorous as those used in the initial treatment, and therefore, the resulting observations should be interpreted accordingly.

Results and discussion

First treatment

Several challenges were encountered during our initial efforts to eradicate zebra mussels from Highline Lake, particularly timing of the treatments during colder winter and spring water temperatures. The detection of an isolated zebra mussel population in late fall 2022 presented a window of opportunity for targeted eradication due to the reservoir's hydrologic conditions. The reservoir's manageable water volume and inputs, coupled with the seasonal closure of the Government Highline Canal, which limits inflow and outflow in the winter, made this possible. During this stagnant water period, it was hypothesized that effective treatment could be achieved without the complication of water exchange typical in the summer and fall when the canal is running, and the amount of water in the reservoir was decreased to enable a more effective molluscicide application while minimizing the required chemical volume. Another factor allowing our probability of success to increase, is that Highline Lake is owned and operated by the state of Colorado, with potential complications with land owners, access, and application logistics being significantly reduced.

Studies have shown that the toxicity of copper, the active ingredient in EarthTec QZ, is both temperature and exposure-duration dependent (Mlouka et al. 2019). While water volume and exposure duration were able to be controlled, water temperatures throughout the treatment remained suboptimal for effective EarthTec QZ application (Rao and Khan 2000). The product label recommends that temperatures during product application should exceed 7 °C for at least 14 consecutive days (Earth Science Laboratories Inc. 2022). In our study, the first application occurred when temperatures exceeded 7 °C for two days, the second for three days, and the third application for ten days (Figure 2). Across all applications, average temperatures ranged from 4.2 °C to 8.7 °C and varied significantly, which indicated a temperature increase over the application periods ($F_{2,394} = 594.8$, $p < 0.001$; Figure 3A). Lower temperatures are known to lead to decreased metabolic rates in mussels, which can lead to decreased ventilation and in turn, decreased metal accumulation, inhibiting oxidative damage to induce mortality and metabolic processes (Heugens et al. 2001; Res et al. 2008). In previous studies, the amount of EarthTec QZ required to induce 100% mortality was significantly higher at 7 °C compared to 12 °C treatments and required double the concentration of product needed at lower temperatures (Mlouka et al. 2019). It is suggested that lower temperatures reduce the effectiveness of copper-based treatments and may affect the physiologic responses of mussels by slowing their metabolism (Calabrese et al. 1984; Banni et al. 2014; Attig et al. 2010; Dondero et al. 2011). The suboptimal temperature in our study likely led to reduced copper toxicity to zebra mussels by reducing respiration and metabolic rates, thereby decreasing the efficacy of our copper treatment (Rao and Khan 2000).

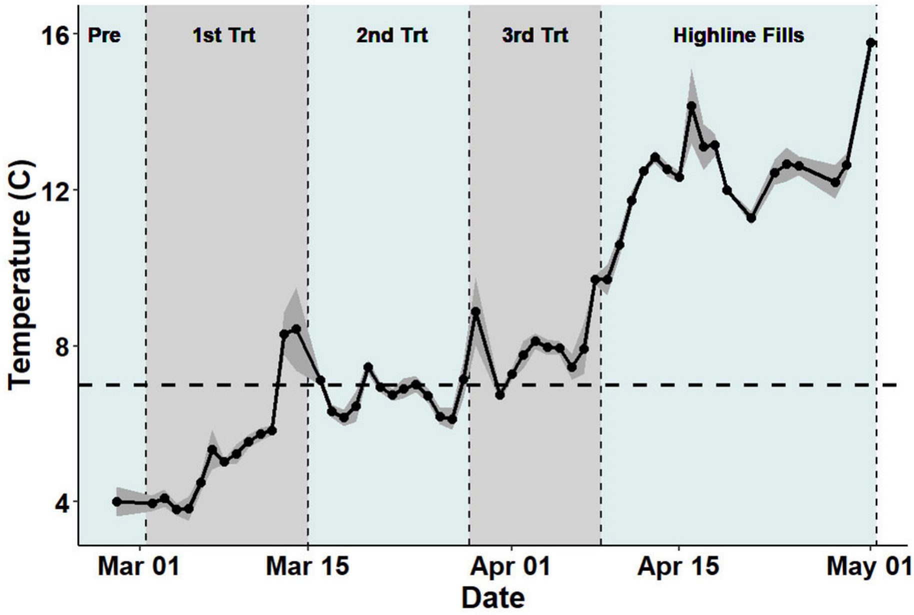

Figure 2. Average temperatures (°C) at Highline Lake collected from all sites prior to treatment (pre), during the first (1st Trt), second (2nd Trt), and third treatment (3rd Trt), and while Highline Lake filled to capacity in 2023. The dashed horizontal line indicates 7 °C which is the lowest temperature recommendation for successful eradication efforts. The grey shaded area around the line represents the standard deviation for each data point.

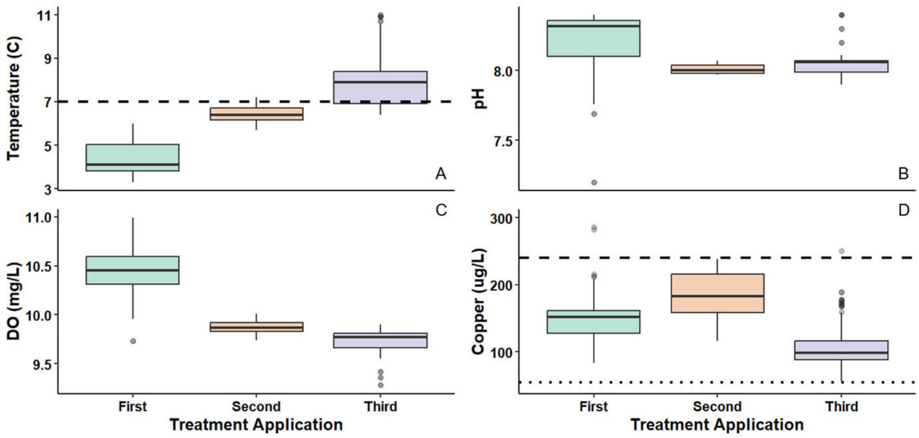

Figure 3. Boxplots of (A) water temperature (°C), (B) pH, (C) dissolved oxygen (DO; mg L^{-1}), and (D) copper concentrations (ug/L) for each of the three treatment periods. The dashed line in (A) represents the 7°C desired water temperature for application. Dashed line in (D) represents the target application concentration of 240 µg/L dissolved Cu based on hardness of 356.0 ± 34.1 mg/L $CaCO_3$ and the dotted line represents the predicted final acute value of 54.0 µg/L from the Biotic Ligand Model based on water quality conditions. Boxplot extent ranges represent the 25th and 75th percentile; band near the middle of the box represents the 50th percentile/median; whiskers range from the lowest to the highest datum with points as outliers.

Previous research has shown that dissolved copper concentrations of 41 µg L^{-1} can lead to 50% reduction in mussel filtration and metabolism, with complete cessation and mortality occurring at concentrations around 100 µg L^{-1} (Kraak et al. 1994). In our study, significant variation in copper concentration was observed among the three applications ($F_{2,394}$ = 193.7, p < 0.001; Figure 3D), but remained higher than 41 µg L^{-1}. During the first application, dissolved copper concentrations ranging from 127.3 µg L^{-1} to

164.6 μg L^{-1} were observed across various depths, with higher concentrations observed in shallow areas. In the second application, copper concentrations in shallow areas were increased, peaking at 193.9 μg L^{-1}, while increased copper concentrations, averaging 189.8 ± 31.0 μg L^{-1}, were also exhibited at deeper sites (3 m or greater). By the third application, copper concentrations were lower, ranging from 95.2 μg L^{-1} to 114.5 μg L^{-1}. Overall, an average dissolved copper concentration of 148.95 ± 43.46 μg L^{-1} was found across all three applications (Figure 3D).

It is important to note that observed toxicity is closely related to copper bioavailability, which cannot be adequately predicted by total or dissolved copper concentrations alone. Instead, it is more accurately correlated with the concentration of free ionic Cu^{2+} (Waite and Morel 1983). Therefore, the quantification of copper bioavailability was essential for accurately predicting the potential toxicity of the copper molluscicide to the mussels in Highline Lake. In general, high water hardness decreases copper toxicity thus, hardness-based criteria for copper toxicity has been used, but has been known to under or overestimate copper toxicity. In 2007, the EPA published the BLM replacing the hardness-based criteria which can still incorporate site based parameters such as hardness and DOC that can affect copper bioavailability. The BLM is a chemical speciation model that incorporates site-specific water chemistry parameters to estimate the dissolved copper concentrations that could lead to toxicity (HDR HydroQual 2016). In this model, copper toxicity is defined as the amount of metal required to cause accumulation at the biotic ligand, a discrete receptor on the organism, where accumulation leads to acute toxicity (Playle et al. 1993). Using the BLM, and incorporating the very hard water values in Highline Lake (356.0 ± 34.1 mg L^{-1} $CaCO_3$; Table S2), with an average DOC of 2.6 ± 0.2 mg L^{-1} during the treatment, and negligible dissolved copper levels prior to treatment of 2.05 ± 0.08 μg L^{-1}, the dissolved copper concentration that might cause toxicity to zebra mussels in our study was estimated. The BLM-predicted FAV was estimated at 54.0 μg L^{-1} dissolved copper, which is the predicted copper concentration likely to cause acute toxicity to sensitive aquatic species.

Toxicity of copper is predicted by the BLM within a factor of ± 2 μg L^{-1} when the model is trained with water toxicity data for fish and daphnia species (Wang et al. 2009b). Few studies have attempted to use the BLM to predict copper toxicity to mussels, and while the model has proven useful, deviations between predicted acute toxicity values and actual acute toxicity values from laboratory experiments have shown that the model tends to under predict toxicity for mussels (Arnold et al. 2005; Wang et al. 2009b). This inaccuracy indicates that the 54.0 μg L^{-1} FAV estimate may be lower than the actual acute toxicity level for the zebra mussels in Highline Lake. Follow-up laboratory experiments to understand dissolved copper toxicity to zebra mussels with high water hardness, low temperatures, and low DOC should be considered.

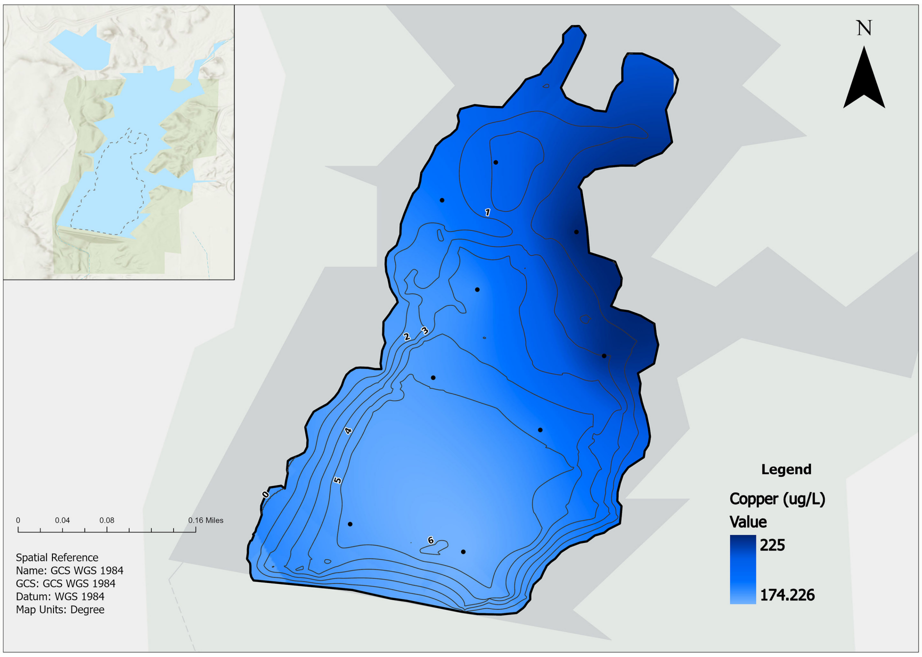

Figure 4. Results from the Kernel Density Model used to predict average dissolved copper concentrations for all three treatments of EarthTec QZ applications across Highline Lake. Contour lines represent the reservoir depths (m) with associated values and black points represent the water sampling locations.

The label requirements for EarthTec QZ allowed for the treatment of only 50% of the reservoir's water body at any given time. Our methods included applying the product only around the perimeter of the reservoir where mussels were most likely to colonize based on their preference for depths less than 6 m, leaving the middle and deeper sections untreated. The target dissolved copper concentration for the first treatment was 4 ppm (240 µg L^{-1} copper), based on the high hardness of Highline Lake, which increases the potential for copper to bind to organic and inorganic substances, thereby reducing its bioavailability. To evaluate copper concentrations throughout the reservoir, and assess mixing of the product without actually applying at various depths, the water was sampled throughout the reservoir and these values were used to estimate copper concentrations at unmeasured sites using a geostatistical model. Model estimates indicated that dissolved copper concentrations remained relatively high (greater than 100 µg L^{-1}) at non-sampled locations throughout the reservoir during the three applications in February, March, and April 2023 (Figure 4), and we have no concerns that the product did not mix in areas of the reservoir that were not treated. Dissolved copper concentrations above 100 µg L^{-1} have been shown to induce 100% mussel mortality in laboratory experiments (Kraak et al. 1994). Our concentrations remained above 100 µg L^{-1} and were higher than the BLM predicted FAV of 54.0 µg L^{-1}, indicating the concentration of dissolved copper was high enough to induce mussel mortality. Higher concentrations were observed in the shallow, muddy flats in the northeastern corners of the reservoir, while copper levels were lower in deeper areas, particularly near the southwestern corner adjacent to the dam (Figure 4). DOC levels remained consistent at 2.6 ± 0.2 mg L^{-1} throughout the treatment.

Average pH values of 8.12 ± 0.17 varied with a significant difference among applications. Reservoir pH was significantly higher during the first application compared to the second and third applications ($F_{2,394}$ = 110.7, p < 0.001; Figure 3B). Dissolved oxygen (DO) concentrations averaged 10.08 ± 0.36 mg L^{-1}, with significant variation across the three applications, and a decline in DO over time, was expected with increasing temperatures ($F_{2,394}$ = 843.7, p < 0.001; Figure 3C). These values are above the required threshold for mussel survival and copper toxicity was likely not affected (Wang et al. 2009a).

After the treatment and as the reservoir began to receive water and fill in April 2023, DOC levels increased to 3.96 ± 0.6 mg L^{-1}, indicating some organic matter enrichment. Average temperatures during the post-treatment phase ranged from 11.4 to 16.2 °C. Dissolved copper concentrations steadily decreased to 43.5 ± 35.2 μg L^{-1} after two days due to dilution, and were below the Colorado acute aquatic life standard (44.5 μg L^{-1}; average hardness 356.0 mg L^{-1} $CaCO_3$). As the water spilled over the dam it was further diluted to 2.4 ± 0.5 μg L^{-1} which was below the chronic aquatic life standard of 26.5 μg L^{-1}; average hardness 356.0 mg L^{-1} $CaCO_3$.

The use of sentinel cages to monitor the mortality of a non-target species, the Asian clam, which served as sentinels for assessing zebra mussel mortality, was inconclusive. Although some Asian clams showed signs of mortality, the cage design and placement failed at six out of nine sites. As a result, it was not possible to directly determine mortality from the copper treatment in all cages. Average mortality (± SD) in the three cages that did not malfunction was 67 ± 30.6%. No clam mortalities were observed in the control sentinel cages located below the dam in Mack Wash. Although we observed approximately 67% mortality in the clams over the three applications, the experimental design and cage placement may have affected the accuracy of these results. The cages detached from buoys or weights, causing them to float rather than remain submerged throughout the treatment period. Additionally, a small number of fish mortalities (fewer than 40 fish), primarily shad and black crappie, were also observed around the reservoir's perimeter, but no major fish kills occurred. In future work, conducting bioassays prior to treatment to assess the sensitivity of zebra mussels and other non-target species to EarthTec QZ under site-specific conditions would be useful. These bioassays would provide a more accurate understanding of the potential effects of copper exposure in the reservoir's specific temperature and water chemistry. Furthermore, improving the design and placement of sentinel cages to better replicate natural substrate conditions would enable more reliable assessments of treatment effectiveness on zebra mussels.

Second treatment

During the second treatment, undiluted EarthTec QZ was applied to the reservoir via boat application, in a manner consistent with the first application during the first treatment, and by dispensing the solution directly from intermediate bulk containers from the shore. All safety protocols were followed as described above. Product dispersion throughout the reservoir was confirmed by water quality samples, and average dissolved copper concentrations of 384.79 ± 38.62 μg L^{-1} were measured with a Hach DR900 colorimeter using the porphyrin method (Method 8143). The water temperature of the reservoir averaged 8.87 ± 0.98 °C for 23 days after application, which may have increased bioavailable copper toxicity during the treatment given the potential for temperature-dependent toxicity above 7 °C. Five sentinel cages, each with five zebra mussels that were collected from the reservoir prior to treatment, were distributed throughout Highline Lake. Some modifications were made to the cages which included more weights to keep the cages submerged in the water and they were capped with threaded PVC caps. In the weeks post-treatment, several of the mussels had detached from surfaces in the cages and closed their shells, presumably a sign of stress (Borcherding 2006). After one month, all mussels detached from the surface, were found in the cages with shells open, and were considered dead. No control cages were deployed to limit the introduction of zebra mussels in waters outside of the reservoir, but mortality seen in the cages suggests copper toxicity to the mussels. Post-treatment monitoring through April 2024 showed that dissolved copper concentrations decreased slightly and as water began to fill the reservoir, concentrations declined below both agricultural and aquatic life standards before the water began to spill over the dam.

In 2024, no detections of live veligers or adult mussels were observed in Highline Lake. However, in July 2024 the presence of veligers was confirmed via microscopy and qPCR in three plankton tow samples from the Government Highline Canal upstream of Highline Lake, in three samples from the Colorado River upstream of Highline Lake and one downstream. Throughout the remainder of 2024, significant sampling efforts were conducted in the Colorado River, Government Highline Canal, and surrounding water bodies. A single positive eDNA detection in the Colorado River downstream of the diversion into the Government Highline Canal was observed after this extensive sampling in the fall of 2024, though no additional veligers or adult mussels were confirmed. The reservoir was completely drained in November 2024, and five dead adult zebra mussels were found during post-draining inspections. The reservoir remained dry over the winter to maximize desiccation and exposure to freezing temperatures. While the retreatment appeared more effective, collateral impact on non-target species was observed, including fish mortality. The mortality of several hundred fish, primarily shad, carp, and white suckers, was likely due to the high concentrations of copper, which is known to be lethal to fish at elevated levels (Figure S1; Johnson 2018). Given the planned eventual draining of the reservoir, fish mortality during the treatment was not the primary concern as a complete loss of the fishery was anticipated. To mitigate the potential spread of invasive species, fish salvage to other waters was not conducted prior to the treatment. However, a "public salvage" initiative was implemented, with bag and possession limits removed to allow anglers to harvest as many fish as possible, thereby reducing resource loss before the application of EarthTec QZ. Additionally, 22 moribund fish were collected to test their filets for copper concentration. Filets were tested at ACZ Laboratories, Inc. to ensure they were safe for public consumption before the fish salvage occurred. Copper concentrations in fish tissues were found to be very low or negligible. The public consumption of all fish was approved by the CDPHE toxicology team, responsible for determining Colorado state fish consumption advisories, after reviewing the results.

Current status

The second treatment, followed by the reservoir's draining to expose mussels to freezing and desiccation, likely eradicated the previous zebra mussel population. Highline Lake was refilled in May 2025 from water diverted from the Colorado River. Following the refill, comprehensive zebra mussel monitoring efforts, including shoreline surveys, plankton tows for veligers, and rigorous boat inspections, were conducted. As of July 2025, two veliger detections were confirmed in plankton tow samples collected from Highline Lake. These detections were initially identified by CPW Aquatic Nuisance Species (ANS) staff at the CPW ANS laboratory and subsequently confirmed via qPCR at CPW's Aquatic Animal Health Laboratory. Furthermore, additional veliger detections were recorded upstream of Highline Lake in the Colorado River after the reservoir's refilling. Although reinfestation was a concern given upstream detections in 2024, the refilling of the lake was unavoidable. This suspected upstream population may have contributed to the reinfestation of Highline Lake post-filling; however, the direct source of this reinfestation remains unconfirmed.

Conclusions

Optimizing treatment timing to coincide with warmer temperatures, ideally above 7 °C, should be a key consideration for future applications of EarthTec QZ or similar molluscicides, when application timelines allow. Despite the challenges posed by low temperatures during our initial treatment of Highline Lake, it was successfully demonstrated by our study that copper from EarthTec QZ can be effectively distributed throughout a large reservoir to achieve concentrations capable of targeting zebra mussel toxicity (54.0 ug L^{-1}

FAV; 100.0 ug L^{-1} Kraak et al. 1994). Our application methods were proven to be both sufficient and safe, with the product's effective deployment being demonstrated without compromising the safety of the crew or equipment, and with minimal boat corrosion being observed during both the first and second treatment.

The resurgence of zebra mussels in Highline Lake during the summer and fall of 2023 highlights that the first eradication effort was not entirely successful. However, it is highly probable that a significantly greater abundance of mussels would have been observed had the reservoir remained untreated by the initial copper-based treatment in February and March 2023. The second treatment, which involved double the concentration of EarthTec QZ and the draining of the reservoir, was considered promising and likely eradicated the mussels from Highline Lake. However, the subsequent detection of mussel veligers in 2025 unfortunately confirmed a reinfestation. The source of the zebra mussels, whether from an existing upstream population or from Highline Lake itself remains unclear. However, a risk of future introductions is presented by the upstream detections made in July 2024 and 2025, posing a substantial challenge to long-term treatment effectiveness. A fundamental limitation of delineation sampling efforts is the inherent difficulty in pinpointing population sources within large, interconnected water systems, especially in a headwaters state. Detecting zebra mussels upstream of a reservoir in headwater regions is challenging because their microscopic larvae are hard to detect, and the environmental conditions may not favor large, easily visible adult populations, making early detection difficult. This challenge complicates decision-making regarding treatment with necessity. Therefore, exploring alternative methods to improve eradication success, such as bioassays or laboratory studies is crucial. Additionally, proactive monitoring efforts must be extended beyond individual water bodies to effectively assess and mitigate potential upstream sources of zebra mussels. Colorado Parks and Wildlife's expanded prevention strategy, including mandatory watercraft inspections at ports-of-entry, enforcement of the clean-drain-dry protocols, and enhanced public education, will continue to be vital in reducing the introduction of invasive species like zebra mussels.

Author's contribution

All authors contributed to research conceptualization. TBRF, RW, BF, TRS, KAM contributed to sample design and methodology. RW, BM, BF, TRS, KAM contributed to investigation and data collection. TBRF, RW, BF, TRS, KAM, MBM contributed to data analysis and interpretation. RW, BF, MKM contributed to ethics approval. RW AND BF provided funding provision. TBRF prepared the original draft of the manuscript. All of the authors contributed to conception and design, acquisition of data, revision of the manuscript, and final approval of the manuscript for submission.

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Ethics and permits

The application of EarthTec QZ in Highline Lake was permitted under the Colorado Discharge Permit System General Permit number CO860000 and CPW's certification number COG860013. The pesticide application was performed in compliance with the EarthTec QZ label under the supervision of licensed pesticide applicators by the Colorado Department of Agriculture as Qualified Supervisors in category 108-Aquatic Pest Control. At the conclusion of the treatment, all reporting requirements specified in the General Permit were satisfied.

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Websites, online databases and software

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Supplementary material

The following supplementary material is available for this article:

Table S1. Calibration standards and respective detection wavelengths for ICP-OES method to detect aluminum, arsenic, calcium, cadmium, copper, iron, potassium, magnesium, manganese, sodium, lead, selenium, and zinc.
Table S2. Results of measured metals from water samples collected before treatments of EarthTec QZ, during the treatment, and after the treatment.
Figure S1. Survival estimates from a bioassay testing various concentrations of dissolved copper from EarthTec® QZ on Asian clams, green sunfish, largemouth bass, speckled dace, and white sucker.

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