INVASIVESNET
Management of Biological Invasions (2021) Volume 12, Issue 1: 85–95

Special Issue: Proceedings of the 21st International Conference on Aquatic Invasive Species
Guest editors: Sarah Bailey, Mattias Johansson, Brenda Koenig and Linda Shaw

Short Communication

Efficacy of low-dose EarthTec® QZ treatment for the control of New Zealand mud snails *Potamopyrgus antipodarum* in a hatchery environment

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Co-Editors' Note: This study was contributed in relation to the 21st International Conference on Aquatic Invasive Species held in Montreal, Canada, October 27–31, 2019 (http://www.icais.org/html/previous21.html). This conference has provided a venue for the exchange of information on various aspects of aquatic invasive species since its inception in 1990. The conference continues to provide an opportunity for dialog between academia, industry and environmental regulators. This paper was not presented at the Conference but contributes to the goal of information exchange.

Citation: Oliver DC, Loubere AD, Sorensen JA (2021) Efficacy of low-dose EarthTec® QZ treatment for the control of New Zealand mud snails *Potamopyrgus antipodarum* in a hatchery environment. *Management of Biological Invasions* 12(1): 85–95, https://doi.org/10.3391/mbi.2021.12.1.06

Received: 31 January 2020
Accepted: 8 May 2020
Published: 3 July 2020

Handling editor: Mattias Johansson
Thematic editor: Sarah Bailey

Abstract

The New Zealand mud snail (NZMS) *Potamopyrgus antipodarum*, is an invasive species of freshwater snail that has become established in the United States. Where they establish, NZMSs can achieve very high densities and have negative ecological impacts. The discovery of NZMSs at Page Springs Hatchery (PSH) in 2019 triggered a need for treatment options in a hatchery that would not result in eradication of fish stocks. The effects of a low-dose (30 ppb) treatment of EarthTec® QZ on NZMSs, Page springsnails *Pyrgulopsis morrisoni* and pond snails *Physella virgata* were evaluated for 39 days. Pond snails fell to zero live individuals 15 days before NZMSs, which fell to zero live individuals 36 days into treatment. Page springsnails fell to zero live individuals three days after NZMSs. It appears that EarthTec® QZ may be an effective treatment for NZMSs under the conditions tested within PSH. Additional testing needs to be performed to evaluate the potential effects on other non-targets under varying environmental conditions.

Key words: copper, trout, springsnails, aquaculture, Arizona, molluscicide, toxicity

Introduction

The New Zealand mud snail (NZMS) *Potamopyrgus antipodarum*, is an invasive species of freshwater snail that has become established on several continents, including North America (Vinson 2004; Cross et al. 2010; Levri et al. 2014). Rapid growth rate, high reproductive potential and tolerance for a variety of aquatic environments makes this species well-suited for expansion to new watersheds (Alonso and Castro-Díez 2008; Levri et al. 2014). Where they establish, NZMSs can achieve very high densities (> 500,000 snails/m^2), alter food webs by sequestering large amounts of primary production (Hall et al. 2003, 2006), and out-compete native grazers (Larson and Black 2016). NZMSs can represent a potential trophic dead end, with sequestered primary production being largely unavailable to predators such as trout due to indigestibility (Vinson and Baker 2008).

NZMSs have been present in the Arizona portion of the Colorado River below Glen Canyon Dam since 1995 (Cross et al. 2010) with downstream dispersal and colonization throughout the system to Lake Mohave by 2008

(Rosen et al. 2012). However, they were not known to be in any other Arizona waterbody until their discovery in two raceways at Page Springs Hatchery (PSH) in 2019. PSH is Arizona Game and Fish's largest hatchery facility dedicated to the production of rainbow *Oncorhynchus mykiss* and brown trout *Salmo trutta* that are stocked throughout the state. Preventing the spread of NZMS (via hatchery trucks and personnel) within and from PSH is uniquely challenging, given that the hatchery produces ~ 57% of all stocked trout in Arizona and makes ~ $185 million positive impact on the state's economy (AZGFD 2015). Given the potential for NZMSs to spread to new watersheds as a result of stocking activities, managers sought a water soluble treatment that could be applied directly to raceways to eliminate NZMSs with minimal harm to trout and other non-target species.

QZ (EarthTec® QZ, Rogers, AR) is a copper based molluscicide currently marketed for treating infestations of Dreissenid mussels. According to the product summary, the molecular structure of QZ (copper sulfate pentahydrate combined with a base acid) maintains copper in the cupric ion form (Cu^{2+}), rendering it more biologically available and less likely to precipitate out of the water column (https://earthtecqz.com/; Hammond and Ferris 2019). Cupric ions bind to negatively charged organic molecules within the cell and prevent normal function of biologic processes (Watters et al. 2013), making Cu^{2+} lethal to almost all aquatic life forms. QZ has been used successfully on both quagga *Dreissena bugensis* and zebra *Dreissena polymorpha* mussels (Iwanyckyj et al. 2017; Watters et al. 2013; Hammond and Ferris 2019) in both laboratory and lake settings. QZ has also been shown to be toxic to gastropod embryos and has potential as a means to control invasive faucet snails *Bithynia tentaculata*; (Carmosini et al. 2018). However, these studies were performed in warm water systems, and effects to non-target species were not assessed quantitatively. Furthermore, concentrations used to achieve 100 percent mortality of target species in previous studies (> 1 ppm) would also be lethal to trout (Howarth and Sprague 1978; Laurén and McDonald 1986; Hansen et al. 2002). Without an effective treatment, complete eradication of the stock and sanitation of the hatchery was the only alternative solution, representing a major monetary loss for the state of Arizona. With preservation of hatchery stock being a major concern, the following objectives were identified for this study:

1. Assess the survivorship of NZMSs exposed to EarthTec® QZ at a dose that is sub-lethal to rainbow and brown trout in a hatchery environment.
2. Assess the survivorship of two non-target, native snail species exposed to the same dosage of QZ in a hatchery environment.
3. Based on the previous two objectives, determine the viability of a chronic, low-dose treatment of EarthTec® QZ as a control for NZMSs while limiting impacts to hatchery stock and non-target species.

Materials and methods

Study Area

Page Springs Hatchery, located in central Arizona, was acquired by Arizona Game and Fish Department (AZGFD) in 1934 and was subsequently renovated in 1991 with increased biosecurity measures. However, PSH is a multi-spring-fed, flow-through hatchery, and these springs could potentially serve as a vector for pathogens and invasive species into the hatchery. In October of 2019, NZMSs were found residing in several raceways at the hatchery (2 of 36 raceways). Additionally, NZMS environmental DNA (eDNA) was detected at multiple locations within the hatchery. PSH is also home to two native species of snail; the pond snail *Physella virgata* and the Page springsnail *Pyrgulopsis morrisoni* which are found in raceways and their associated infrastructure. Page springsnails are listed by the state of Arizona as a species of greatest conservation need (AZGFD 2012).

Study Design

In addition to evaluating the effect of QZ on NZMSs, we evaluated survivorship of the two native species of snail found at PSH. Page springsnails, like NZMSs have an operculum that can be used to segregate themselves from the environment (Martinez and Thome 2006; Levri et al. 2007). Pond snails lack an operculum and thus may be more susceptible to toxicity from QZ relative to the other two species.

QZ, delivered via metered dispersal (i.e., measured release of chemical relative to water flow), was mixed with source spring water prior to entering the raceways to ensure the most consistent and evenly mixed concentration of copper (Netherland et al. 1998). All raceways received the same mixture of QZ and spring water. A target Cu^{+2} concentration of 60 µg/L (ppb), approximately 25% of a 240 ppb lethality threshold for rainbow trout (Johnson 2018), was attempted. However, after the first 12 hours, Cu^{+2} concentrations were reduced to approximately half due to extensive rainbow and brown trout mortality (> 100,000 fish died); fish mortality did not stabilize until 6 hours post-concentration reduction. The lower (30 ppb) Cu^{+2} concentrations were checked (~ 3 times daily via a spectrophotometer) and maintained throughout the remaining duration of the study. As a result, the treatment for this study was a 60 ppb for 12 hours followed by 30 ppb concentration for the subsequent 38.5 days.

A split-plot study design was used to allow for the three species of snail to be evaluated simultaneously (Lawson 2014). Specifically, the study was conducted in six replicate raceways with replicates receiving two sub-replicate (i.e., repeats) mesh containments for each of the three species (Figure 1). Ten individual snails collected from raceways prior to initiation of treatment were placed in each of the sub-replicate mesh containments

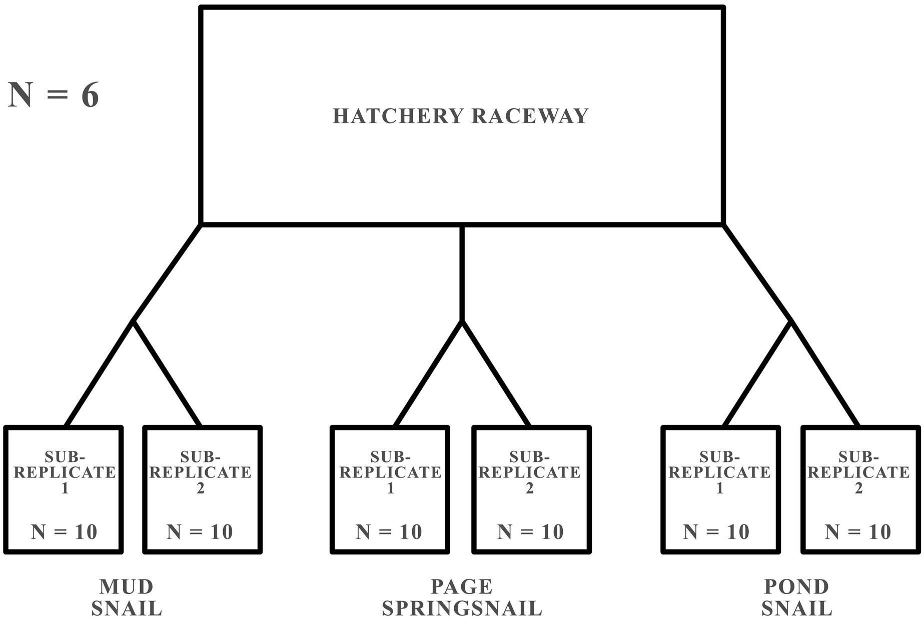

Figure 1. Diagram of the split-plot study design.

(N = 20 per raceway per species; N = 120 per species across raceways; Figure 1). Sub-replicate mesh containments were large enough to allow water to flow through it, but small enough to prevent escapement; no escapement was observed. Additionally, a small polypropylene cup was placed inside each sub-replicate containments to provide structure for the snails.

Survivorship Evaluation and Statistical Analysis

Snail survivorship was evaluated every 72-hours following initiation of treatment and was terminated after 39 days. To determine survivorship during an evaluation event, snails were removed from treatment water and placed in non-treatment (freshwater) water to stimulate activity. Freshwater exposure was kept constant across replicates within a sampling period and was consistent across sampling periods (21.46 minutes ± 0.22 SE [Standard Error]). During the freshwater exposure, a dissection microscope was used to count all living snails for each sub-replicate. Snails were considered alive if they were not empty shells, did not demonstrate visible morbidity, and displayed signs of activity. After the freshwater exposure, all snails alive or dead were placed back in their sub-replicate containments and returned to treatment water. All snails were evaluated and counted in every evaluation period.

A generalized linear mixed model (generalized Poisson distribution, log link) was used to assess statistical differences in survivability (i.e., count of living snails) of the three snail species using the glmmTMB package for R (Stroup 2013; Faraway 2016; Magnusson et al. 2019a). The fixed effects of days post treatment (ordinal), species (categorical), and their interaction were used; a nested random intercept term of sub-replicate (categorical) within raceway (categorical) was also included. The random intercept term

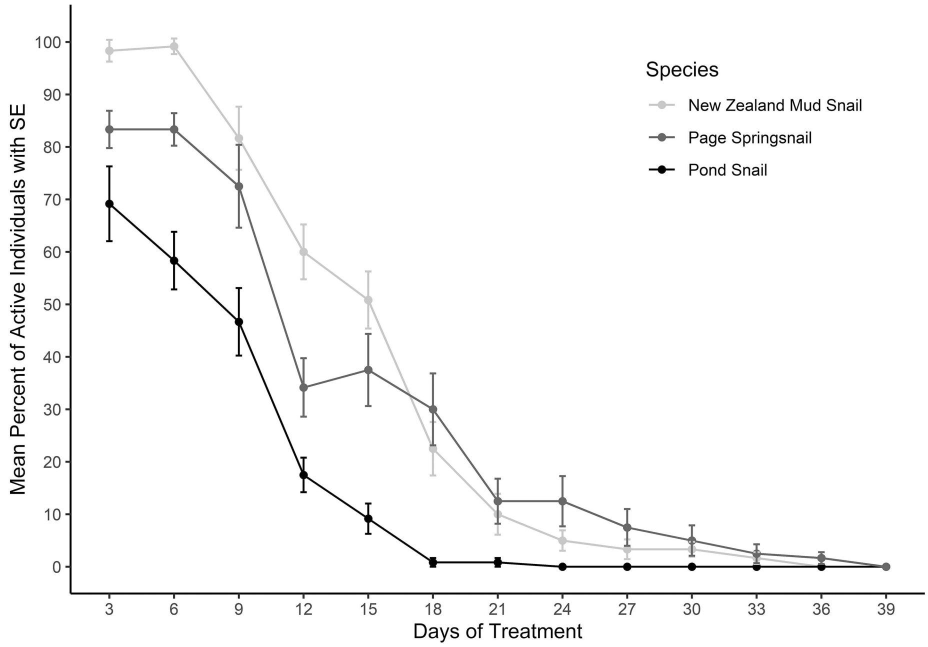

Figure 2. Mean percent of active individuals with standard error (SE) plotted against days of treatment for three species of snail at Page Springs Hatchery.

accounted for variation between raceways and variation between sub-replicates within a raceway. Zero-inflation occurred and was addressed using a zero-inflation formula (binomial distribution, logit link) with a single fixed effect term of species (Stroup 2013; Faraway 2016; Magnusson et al. 2019b). Tukeys adjusted simple-pairwise comparisons (i.e., species across days and across species by day) of the fixed effects were performed using the emmeans package, post-hoc (Stroup 2013; Lenth et al. 2019). All statistical assumptions were assessed and met or addressed, and statistical significance was evaluated at an $\alpha = 0.05$ (Stroup 2013; Murtaugh 2014; Faraway 2016).

Results

Water quality remained consistent throughout the course of treatment. Mean water temperature during the course of treatment was 19.48 °C ± 0.06 SE. Mean pH of raceway water was 7.84 ± 0.10. Alkalinity and hardness (reported in mg/L $CaCO_3$) were 199.00 ± 2.00 and 176.00 ± 6.00 respectively. Concentration of Cu^{2+} was consistent across raceways for the duration of treatment (30.07 ppb ± 0.31 SE) following the reduction in concentrations due to high fish mortality. Trout mortality reduced to normal operational levels following the reduction of Cu^{2+} from 60 ppb to the concentration listed above, and remained at that level for the duration of the study (AZGFD *unpublished data*).

There was no statistically significant drop in survival of the three species from the initiation of treatment until evaluation on the 12th day of treatment ($p < 0.03$, $d.f. = 423$, $t\text{-}ratio = 3.82–6.58$; Figure 2). Mean survivorship (i.e., active living snails) of NZMSs, Page springsnails, and pond snails at the 12th

day of treatment was 60.00% ± 5.20 SE, 34.16% ± 6.80 SE, and 17.50% ± 3.28 SE respectively. Survivorship for NZMSs and Page springsnails did not decline below 50% until evaluation on the 18th day of treatment (Figure 2). Pond snails dropped below 50% survivorship when they were evaluated 12th day of treatment (Figure 2). New Zealand mud snail survivorship fell to 0% when they were evaluated on the 36th day of treatment (0.00 ± 0.00 SE; Figure 2). Page springsnail survivorship fell to 0% when they were evaluated on the 39th day of treatment (0.00 ± 0.00 SE; Figure 2). When pond snails were evaluated on the 24th day of treatment there was 0% survivorship (0.00 ± 0.00 SE; Figure 2).

Statistical differences between species were not observed prior to the evaluation on the 12th day of treatment ($p \geq 0.05$, *d.f.* = 423, *t-ratio* = −2.65–3.61) for any species comparisons within an evaluation. Statistical differences existed between mud and pond snails during the evaluations that occurred on the 12th and 15th day of treatment ($p < 0.05$, *d.f.* = 423, *t-ratio* = 5.50 and 6.20) within their respective evaluations. Statistical differences existed between Page springsnails and pond snails during the evaluations that occurred on the 15th and 18th day of treatment ($p < 0.05$, *d.f.* = 423, *t-ratio* = 3.72 and 4.95) within their respective evaluations. No other statistical differences between species occurred following the evaluation on the 18th day of treatment ($p \geq 0.05$, *d.f.* = 423, *t-ratio* = −2.87–2.68) within any given evaluation.

Discussion

We were unable to establish controls for this study given that the entire hatchery was being treated with QZ, and source water could not be isolated due to the design of the spring boxes. However, study duration did not exceed the life expectancy (mature at 4–6 months) for NZMSs (Tibbets et al. 2010), and the presence of multiple generations of all three snail species, as well as fish and other invertebrates, indicated that raceway conditions would have otherwise been conducive to snail survivorship in the absence of QZ. The lack of controls also does not allow us to separate out the potential impacts of repeated handling. The effects of repeated handling are likely minimal given the short duration of handling time relative to the extent of the study and the tolerance of NZMSs to a variety of harsh conditions including extended air exposure, gastrointestinal tracts for up to 24-hours, and short exposures to quaternary ammonium (Schisler et al. 2008; Bruce et al. 2009; Alonso and Castro-Díez 2012). While the same cannot be said for pond snails and Page springsnails, all three species of snails were also found in the hatchery raceways uncontained (not part of our study) and over the course of the treatment those uncontained snails died off, suggesting that QZ treatment is responsible for snail mortality and not the effect of repeat handling. As a result, we believe that all changes in survivorship are likely a direct response to the application of QZ.

Consequently, we determined that the dosage of QZ used in this study is lethal to all species of snail tested in this study under hatchery conditions. Pond snails were the most susceptible to QZ, and were the first species to reach zero live individuals. In fact, pond snail survivorship fell to 0% 12 days before NZMSs. This result is unsurprising given that pond snails do not possess an operculum, and thus cannot seal their shells and limit their exposure to toxicants in the water like Page springsnails and NZMSs. Page springsnails, which are considered to be fairly sensitive to poor water quality (Martinez and Thome 2006), experienced similar survivorship to NZMSs, with survivorship falling to 0% on the last day of evaluation (39) versus day 36 for NZMSs. A period of low activity around day 12, followed by a period of increased activity on day 15 led to an apparent increase in survivorship of Page springsnails for that evaluation period. This was in part due to the small size of Page springsnails relative to the other two species, which led to difficulty in accurately assessing whether individuals were alive or dead. In anticipation of and to account for this, we retained dead individuals for all three species and continued to count them for the duration of the study rather than removing them from counts. This allowed us to account for error involved with assessment of survivorship of individual snails. Despite a slight bump in survivorship during that time period, survivorship of Page springsnails continued to decline for the rest of the treatment period. The mortality observed during the course of treatment at PSH supports findings in Hammond and Ferris (2019) that a chronic, low dose exposure to QZ is an effective option to treat mollusk infestations. Intuitively, this makes sense, as chronic exposure counters the ability of species such as NZMSs to seal their opercula and isolate themselves from the water until the Cu^{2+} dissipates. We observed 100% mortality in NZMSs within a similar time frame despite using a fraction of the dose delivered to quagga mussels in the Hammond and Ferris (2019) study, indicating that QZ could be an effective control measure against NZMSs. The observed effect on non-target snails may be concerning to managers though, especially given that Page springsnails are a species of special concern with limited range and water quality tolerance (Martinez and Thome 2006). While this study has shown a low dosage of QZ to be effective at inducing mortality in NZMSs, effects to non-target species needs to be considered fully before a decision is made regarding widespread application. While the negative impacts from the proposed concentration of copper may be minimal for some invertebrate taxonomic groups like chironomids, its effects may be more deleterious to other taxonomic groups such as amphipods, oligochaetes, and planarians (Anderson et al. 1980; Timmermans and Walker 1989; West et al. 1993; Knakievicz and Ferreira 2008), and mollusks as demonstrated in this study. Although the presence of these taxa are often minimal in a hatchery setting, the detrimental effects on populations of these taxa in natural systems may have widespread ecological impacts ranging from acute impacts on mobility, behavior and regeneration to long-term developmental abatement and morbidity (Arthur and Leonard 1970; Timmermans and Walker 1989; West et al. 1993; Knakievicz and Ferreira 2008).

The dosage of QZ used in this study was much lower than what has been reported as effective for both Dreissenid mussels and other species of snail. However, exposure times in previous studies (4–7 days) were shorter than in this study and non-target fish species were not a concern (Watters et al. 2013; Iwanyckyj et al. 2017; Carmosini et al. 2018). Conversely, effects were observed on non-target fish species in addition to non-target invertebrate species during this study. Initial concentrations were set at 60 μg/L, approximately 25% of the lethality threshold identified for rainbow trout by Johnson (2018). However, during the first 12 hour period, a considerable increase in rainbow and brown trout mortality was noticed. Thus, the decision was made to reduce the concentration for the duration of the study. This same acute mortality effect was not observed in any of the three snail species. The trout mortality event that occurred as a result of the elevated concentration continued for an additional 6 hours post reduction in QZ; mortality for the first 18 hours exceeded that of the previous 30 days of operation (AZGFD *unpublished data*) for both rainbow and brown trout combined. After the concentration was reduced to ~ 30 μg/L, post the initial 18 hours, trout mortality reduced to normal operational levels for the duration of treatment. The initial dosage was based upon a bioassay of QZ on rainbow trout that EarthTec® commissioned from a third party (Johnson 2018). However, conditions in that assay did not mimic a raceway setting, and water quality factors such as temperature, hardness and alkalinity (12.01 °C ± 0.003 SE, 127.1 mg/L ± 6.542, and 64.5 mg/L ± 0.4539 respectively in the assay) that can potentially affect trout susceptibility to Cu^{2+} were different to conditions at PSH (Howarth and Sprague 1978; Chakoumakos et al. 1979; Watters et al. 2013; Crémazy et al. 2017; Santos et al. 2019). Interestingly, the results reported by the above publications indicate that water at PSH should have increased trout resistance to Cu^{2+} relative to the bioassay. The fact that we reported high mortality at 60 ppb Cu^{2+} indicates that there may be additional factors outside water quality at play. The reported lethal concentration of QZ in the assay did not account for confounding factors such as higher stocking densities in a raceway (relative to a laboratory setting) and other sources of stress that could increase trout susceptibility to toxicants in the water column. While stocking densities did not exceed those considered to be deleterious to trout during this study under normal hatchery conditions (Ellis et al. 2005), the introduction of QZ into the system may increase stocking density-based stress responses. Finally, tolerance of brown trout to QZ has not been assessed at all, leading to concern that brown trout could be more susceptible to copper poisoning based on the observed mortality at the beginning of the study. This is of particular concern at PSH, given that the outflow from the hatchery drains directly into Oak Creek, which is a popular fishing destination and supports a recreationally important population of brown trout. Reduction of the QZ dosage likely extended the treatment period needed to see 100% mortality of NZMSs, but did indicate that there appears to be a dosage of QZ that both brown and rainbow trout can tolerate that is simultaneously lethal to NZMSs. More testing is needed to accurately determine the tolerance limit of rainbow and brown trout to QZ in hatchery raceways. Additionally, the chronic effect of QZ on fish are unknown and further evaluation of its effects beyond the 39 day period evaluated in this study are warranted. Unrestricted, undiluted, or unprocessed (i.e., tertiary treatment or settling ponds) outflow from hatcheries receiving QZ for mollusk control should be cautioned against. While it may be effective in a hatchery setting, the utility of a low-dose of QZ as a control for NZMSs may be diminished in a more natural ecosystem, especially when potential effects to non-target species are taken into account. Toxicity avoidance behavior mechanisms of NZMSs (i.e. burrowing) and increased rates of environmental precipitation of Cu^{2+} are both of potential concern in reducing the effectiveness of a QZ treatment, further evaluation is needed. Finally, further refinement of LC50's (i.e., lethal concertation to achieve 50% mortality) for NZMSs and non-target species (both vertebrate and invertebrate) and the relationships with temperature, hardness, and alkalinity needs to be further investigated as they are likely not consistent within Arizona hatcheries, let alone across the country.

Acknowledgements

We would like to acknowledge the following people for technical and logistic support: Mark Dahlberg, Ryan Mann, and Geoffrey Rabinovich. We would like to thank Dr. David Hammond for providing hatchery staff with instruction on the application of EarthTec® QZ and the testing of copper levels, and for providing us with the third-party bioassay outlining Earthtec® QZ rainbow Trout toxicity. We would also like to thank the following people for assistance with sampling evaluation of snails: Alex Martinez, Emily Higuera, Lorraine Avenetti, and Page Springs Hatchery staff. Additionally, we would like to thank the reviewers for their comments and suggestions.

Funding Declaration

Funding was provided by Sportfish Restoration Act grants. Additionally, chemical was provided by Earth Science Labs Inc. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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