



Evaluating the Life-History Responses of Adult Invasive (*Bithynia tentaculata*) and Native (*Physa gyrina*) Snails Exposed to a Cu-Based Pesticide (EarthTec® QZ)

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Abstract

The faucet snail, *Bithynia tentaculata*, is an invasive snail that facilitates outbreaks of waterfowl disease in the Upper Mississippi River of the United States. In response, there is interest in identifying strategies that mitigate its population and spread. In this study we assessed the effects of a copper (Cu) molluscicide, EarthTec® QZ, at three concentrations (0, 0.1 and 0.6 mg/L Cu) on adult *B. tentaculata* and a coexisting native species, *Physa gyrina*. We found that in the 0.6 mg/L Cu treatment, ~68% of *B. tentaculata* snails remained alive after a 4-day exposure whereas all *P. gyrina* snails died. In contrast, a majority of both snail species remained alive and active after 4 days in the control and 0.1 mg/L Cu treatments. Although *B. tentaculata* demonstrated higher survivorship, it bioaccumulated more Cu than *P. gyrina*. Additionally, examination of *B. tentaculata* individuals revealed that females tended to exhibit higher mortality than males.

Keywords *Bithynia* · *Physa* · Invasive species · Mortality · Bioavailability · Copper

Invasive species are recognized as key threats to freshwater biodiversity and ecosystem stability worldwide (Tricarico et al. 2016). Although numerous invasive taxa are responsible for negatively impacting native species and communities, mollusks have been identified as one of the most detrimental groups due to their consumption of native primary producers (Naddafi et al. 2007), their capacity to reproduce and disperse (Alonso and Castro-Díez 2008), and their potential to harbor and transmit parasites (Sandland and Peirce 2021).

Bithynia tentaculata is an invasive operculate aquatic snail that was introduced into the Great Lakes from Europe in the late 1800s (Mills et al. 1993). It has since spread to freshwaters across North America, including the Upper Mississippi River (Déry et al. 2000; Sandland and Peirce 2021). The occurrence of this species is of concern as it can come

to dominate gastropod assemblages (Weeks et al. 2017) and transmit parasites to waterfowl leading to outbreaks of disease (Sauer et al. 2007). These impacts on local biota have led to interest in developing strategies for controlling the population size and spread of this snail.

EarthTec® QZ is a copper (Cu) molluscicide that delivers the active metal ingredient (Cu²⁺) with proprietary components that reduce its complexation with aquatic ligands, thereby lengthening the time the metal remains bioavailable. Past work has shown that EarthTec QZ can reduce the establishment of nuisance mussel larvae and the survival of adult mussels under semi-natural conditions (Watters et al. 2013). Field applications have also shown promise for controlling mussels in partial- and whole-lake applications (Hammond and Ferris 2019; Lund et al. 2017). In terms of gastropods, Carmosini et al. (2018) showed that EarthTec QZ reduced the survival of *B. tentaculata* embryos and that certain application regimes had greater impacts on this invasive snail compared to a native pulmonate gastropod [*Physa* (= *Physella*) *gyrina*]. Although these results suggest that EarthTec QZ may aid in controlling *B. tentaculata*, more information is required on its effects towards additional snail life-stages in both invasive and native species.

In this study, we used a comparative approach to assess the sensitivities of adult invasive (*B. tentaculata*) and native

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(*P. gyrina*) snails to EarthTec QZ. We also compared survival between male and female *B. tentaculata*. In a follow-up experiment, we determined Cu bioaccumulation in each species by quantifying Cu in snail tissues.

Materials and Methods

EarthTec QZ, provided by the manufacturer (Earth Science Laboratories, Rogers, AR), delivers Cu^{2+} as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (19.8%) along with proprietary ingredients (80.2%); thus the concentration of active Cu^{2+} in the product is 5%. Dosing solutions (0.1 and 0.6 mg/L Cu) were prepared in filtered well water ($<0.45 \mu\text{m}$, $\text{pH} = 7.84 \pm 0.01$, alkalinity = $227 \pm 3 \text{ mg CaCO}_3/\text{L}$, specific conductivity = $471 \pm 1 \mu\text{S/cm}$). These concentrations reflect manufacturer-recommended low and medium, 4-day treatments for quagga and zebra mussels. Actual Cu concentrations were determined by Microwave Plasma-Atomic Emission Spectroscopy (Agilent Technologies 4100 MP-AES). There was no detectable Cu in the original filtered well water (LOQ = $10 \mu\text{g/L Cu}$).

Adult *B. tentaculata* and *P. gyrina* snails were collected on 22 May 2018 from Mississippi River Navigation Pool 8 located between lock and dam 7 (Campbell, WI) and 8 (Genoa, WI). *Bithynia tentaculata* is a dioecious operculate snail that feeds via radular scraping and/or filter feeding; adults can reach a maximum shell length of 10–12 mm at our site. Conversely, *P. gyrina* is hermaphroditic pulmonate species and feeds primarily by scraping food from the substrate. In Pool 8, adults of this species have similar shell lengths (10–15 mm) to those of *B. tentaculata*. Collected snails were placed into species-specific polyethylene terephthalate (PET) cups (266 mL; 6 to 10 snails/cup) with filtered well water and acclimated for a minimum of 9 days during which time they were fed lettuce ad libitum and water was refreshed every 2 days. Cups were maintained at 20°C and exposed to a ~14 h light: 10 h dark photoperiod.

For the toxicity assay, size-matched snails were individually allocated to 17-mL polystyrene (PS) cell-culture wells (Corning™ Costar™) containing 8 mL of either 0, 0.1, or 0.6 mg/L Cu solution (36 snails/treatment/species). Wells were covered with PS lids to minimize evaporation and incubated at 20°C on a 14 h light: 10 h dark photocycle for 4 days to mimic conditions at the collection site. Snails were not fed during the 4-day exposure. Individuals were assessed every 24 h and were recorded as dead if they did not respond to touch by a dissection probe. If snails were alive, general behaviors (such as whether they were actively moving, stationary, or retracted into their shells) were noted in a binary fashion (i.e., either “yes” or “no”). Since *B. tentaculata* is a dioecious species (whereas *P. gyrina* is hermaphroditic), individuals were sexed upon death or at the end of the experiment.

To assess Cu bioaccumulation, snails were collected from Pool 8 on 23 July 2018, as was river water for Cu analysis. In the lab, snails were acclimated, 0, 0.1 and 0.6 mg/L Cu treatment solutions were prepared, and a 4-day exposure was conducted as described for the toxicity assay (above) with 6 snails/treatment/species. Snails that died during the experiment were transferred to polypropylene centrifuge tubes and immediately frozen. At the end of the 4-day exposure, remaining living snails were similarly preserved.

To determine Cu accumulation in individual snails, soft tissues were separated from the shell, freeze-dried (Virtis DBT BenchTop Freeze Drier), weighed, and digested with 5 mL of a 50:50 HNO_3 (69% w/w trace metal grade, Fisher Chemical) and H_2O_2 (30% w/w, Acros Organics) solution using a CEM Mars 6 Microwave Digestion System with the following conditions: power = 1030–1800 W, ramp time = 20 m, hold time = 15 m, temperature = 200°C . Yttrium (Y; 264 μg ; Agilent Technologies) was used as an internal standard to monitor recovery for the digestion process. Digested samples were diluted with deionized water ($18.2 \text{ M}\Omega \text{ cm}$) to 6.9% HNO_3 and Cu and Y were quantified by Inductively Coupled Plasma—Optical Emission Spectroscopy (Agilent Technologies 5110 ICP-OES; LOQ = $3.2 \mu\text{g/L}$). Lettuce used to feed the snails during acclimation was analysed in the same way. To determine Cu in Mississippi River water, samples were filtered ($<0.45 \mu\text{m}$), acidified (5% HNO_3) and analysed by ICP-OES.

Binary logistic regression was used to investigate snail survival at the end of the 4-day exposure. Snail species (*B. tentaculata*, *P. gyrina*) and Cu concentration (0.0, 0.1, 0.6 mg/L Cu) were both included as main effects in the model. We also used binary logistic regression to explore sex-based differences in *B. tentaculata* survival across different Cu concentrations. Data for Cu in snail tissues was assessed for adherence to parametric assumptions and found to violate the homogeneity of variance assumption; thus data were \log_{10} transformed prior to analysis with two-way ANOVA. Species and Cu concentrations were used as main effects in the model. Data were analysed using SPSS software (version 25); results were deemed significant at $\alpha \leq 0.05$.

Results & Discussion

Due to *B. tentaculata*'s potential impacts on native species, interest has grown in devising strategies for its control. Here we assessed the effects of EarthTec QZ, a Cu molluscicide used for the control of nuisance zebra and quagga mussels, on the survivorship of adult invasive (*B. tentaculata*) and native (*P. gyrina*) snails, and quantified Cu uptake by each species. In general, adult *B. tentaculata* exhibited higher

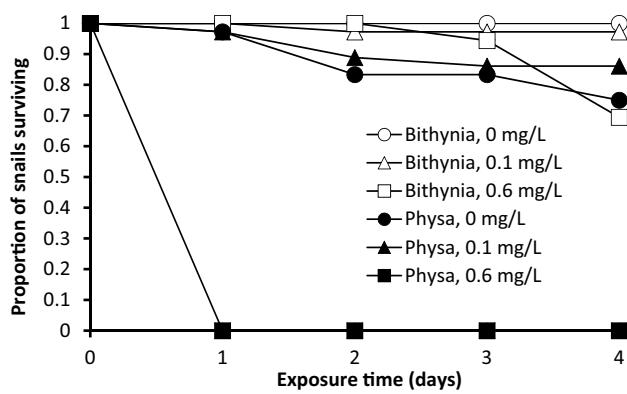


Fig. 1 Proportion of *B. tentaculata* and *P. gyrina* snails surviving over 4 days of exposure to three concentrations of Cu from EarthTec QZ

survival than *P. gyrina*. Differences between species were most pronounced in the 0.6 mg/L Cu treatment where ~68% of *B. tentaculata* were alive after 4 days whereas all *P. gyrina* died (Fig. 1). A majority of both species in the control and 0.1 mg/L Cu treatments remained alive and active during the 4-day exposure. In contrast, both species exhibited immediate stress in 0.6 mg/L Cu. *Bithynia tentaculata* snails retracted into their shells and closed their opercula. Most re-emerged after 48 h but then either died shortly thereafter or remained in a semi-static state. *Physa gyrina* snails in the 0.6 mg/L Cu treatment crawled around the wells rapidly for a short time (minutes to hours) before entering a semi-static state and dying (< 24 h). Binary logistic regression revealed that snail species (Wald = 17.92, df = 1, P < 0.001) and Cu concentration (Wald = 25.23, df = 2, P < 0.001) were good predictors of snail survival.

The trends in survivorship for adult *B. tentaculata* and *P. gyrina* differ from past work that showed EarthTec QZ could eliminate *B. tentaculata* embryos using the manufacturer recommended 0.6 mg/L Cu treatment for nuisance mussels, and that under certain dosing regimes, survivorship and hatching were reduced for the invader compared to the native snail (*P. gyrina*) (Carmosini et al. 2018). Greater Cu sensitivity by embryos relative to adults has also been reported for the pulmonate snail *Lymnea luteola* L. For *L. luteola*, the 4-day EC₅₀ for embryos was 28.31 µg/L Cu whereas for adults the EC₅₀ for a 7-day exposure was more than twice as high at 63.62 µg/L Cu (Das and Khangarot 2011; Khangarot and Das 2010). Furthermore, *L. luteola* adults exhibited 100% mortality after exposure to 0.180 mg/L Cu for 3 days, suggesting that this species is more sensitive to Cu than either *B. tentaculata* or *P. gyrina* (Das and Khangarot 2011). Greater Cu sensitivity of the pulmonate *P. gyrina* compared to the operculate *B. tentaculata* in our study is consistent with work by Arthur and Leonard (1970) who reported 4-day EC₅₀ values of 0.039 and 1.7 mg/L Cu for *P.*

integra (pulmonate) and *Campeloma decisum* (operculate), respectively. The high EC₅₀ for *C. decisum* indicates that this species is quite resistant to Cu and that substantial variation in Cu sensitivity exists among gastropods.

Interestingly, *B. tentaculata* snails survived better even though their tissues accumulated more Cu compared to *P. gyrina* (Fig. 2). Snail tissue Cu across dosing concentrations revealed a significant interaction between snail species and exposure dose ($F_{2,28} = 63.54$, P < 0.001). This was driven by increasing differences in tissue Cu between the species with increasing Cu dose (Fig. 2). The main effects of snail species and Cu dose were also significant (both P < 0.001).

The fact that adult *B. tentaculata* exhibited higher survival than *P. gyrina* was not unexpected, given that the former possesses an operculum that can provide a protective barrier to biotic (e.g., predation) (Kelly and Cory 1987) and abiotic (e.g., desiccation) stressors (Wood et al. 2011) when closed. Indeed, this structure has been identified as a reason why operculate snails can persist through short-term metal exposures better than species lacking the structure (Arthur and Leonard 1970). However, what was surprising were the high Cu concentrations recovered from living *B. tentaculata*, which actually exceeded those that killed *P. gyrina*. In this study, *B. tentaculata* subjected to the high Cu concentration immediately sealed themselves in their shells, but after 3 to 4 days, the head-foot was seen extending out from the aperture in these individuals, presumably leading to Cu accumulation in the soft tissues. These observations suggest that the operculum of *B. tentaculata* may only provide short-term protection from external Cu conditions and that other internal factors, such as the snail’s ability to sequester (Ng et al. 2011) and/or detoxify Cu (Atli and Grosell 2016), may be more important for surviving extended periods of metal exposure. The capacity of *B. tentaculata* to mitigate Cu toxicity through these mechanisms may help to explain

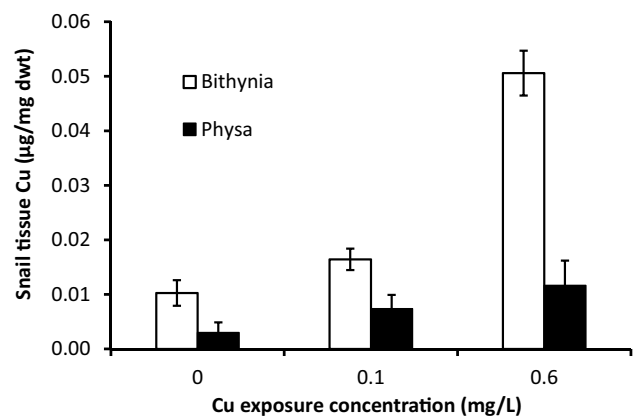


Fig. 2 Mean (± standard error, n=6) tissue Cu concentrations in *B. tentaculata* and *P. gyrina* snails exposed to three concentrations of Cu over a 4-day exposure period

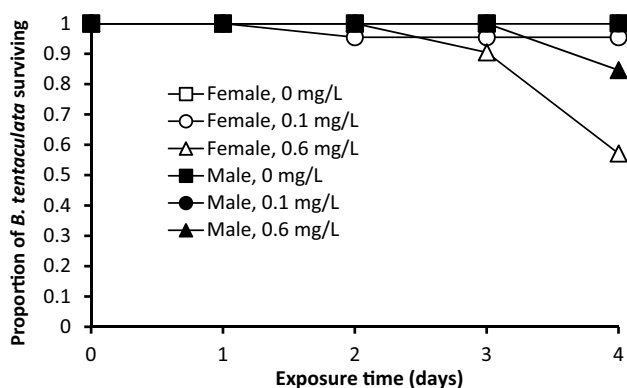


Fig. 3 Proportion of female and male *B. tentaculata* snails surviving over 4 days of exposure to three concentrations of Cu from EarthTec QZ

why this species was better able to survive higher tissue concentrations of Cu compared to *P. gyrina*.

It should be noted that *B. tentaculata* and *P. gyrina* tissues contained Cu even when they were maintained in Cu-free (control) treatments (Fig. 2). The use of the Y internal standard confirmed that our tissue digestion methodology was highly efficient (recovery mean \pm standard deviation = $102\% \pm 2\%$). Copper uptake due to our maintenance procedures is unlikely, as Cu was not detected in either the water used to acclimate snails nor in the lettuce that was fed to them. Snails may have acquired Cu in the field prior to collection, as analysis revealed the presence of Cu in water samples from the collection site ($0.02 \pm <0.01$ mg/L Cu). A more likely explanation is that the background tissue Cu reflects natural concentrations found within the circulatory systems of these snails (Flessas et al. 2000). For many gastropods, oxygen transport occurs via hemocyanin proteins, which contain Cu atoms (Kato et al. 2018). Although the specific hemolymph components of *B. tentaculata* and *P. gyrina* are not known, hemocyanin has been found in closely related species (Suwannatrai et al. 2016; Van Kuik et al. 1987).

Since *B. tentaculata* is a dioecious species, we also compared survival between males and females and found that survival tended to diverge between sexes as Cu exposure concentration increased (Fig. 3). Since there was no difference between male and female *B. tentaculata* in the control treatment, binary logistic regression was restricted to snails exposed to 0.1 and 0.6 mg/L Cu. Not surprisingly, the effect of concentration remained significant and consistent as snails exposed to the lower concentration exhibited higher survival (Wald = 7.15, df = 1, $P = 0.007$). Interestingly, there was also a trend towards higher survival in males compared to females, particularly in the 0.6 mg/L Cu treatment where only ~57% of females survived after 4 days compared to ~86% of the males (Fig. 3); however,

although suggestive, this pattern was not significant at $\alpha = 0.05$ (Wald = 3.07, df = 1, $P = 0.08$).

Sex-based differences in survival and other life-history traits have been reported in both vertebrates (Perkins et al. 1997) and invertebrates (Foley et al. 2019) exposed to Cu. There is evidence suggesting that these differences may be due to variation in the detoxification capacities between males and females (Halmenschelager and da Rocha 2019). Another, non-mutually exclusive possibility is that Cu interacts with sex-specific allocation strategies leading to different life-history expressions (Kramarz et al. 2014). Female *B. tentaculata* used in our experiment were collected during their reproductive peak (May–June). Thus, the energy committed to egg production (embryos plus egg mass components) in females may have limited the resources available for mitigating the physiological stresses associated with Cu exposure resulting in higher mortality compared to males.

Higher mortality in adult *P. gyrina* compared to *B. tentaculata* is opposite to what has been reported for the embryos of these two species (Carmosini et al. 2018). Taken together, these results suggest that *B. tentaculata* embryos would be the preferable target for EarthTec QZ treatment. Practically however, a number of application challenges remain given that both the invasive and native species occupy similar aquatic habitats. In addition, other potentially co-occurring native lymniids and endangered or threatened mussels (e.g., *Stagnicola elodes*, *Quadrula fragosa*), for which Cu^{2+} toxicity data are not available, may also be negatively impacted. Moreover, Cu toxicity has been documented for other common aquatic animals and algae, such as cladocerans, amphipods, and *Chlorella* algal species; thus, the ability of an aquatic ecosystem to recover post-molluscicide treatment must be carefully considered (EPA 2007). Combining our knowledge of Cu susceptibilities for multiple species and developmental stages, along with other life history features, such as the timing of reproductive periods and detailed behavioral responses, may help to refine dosing strategies aimed at maximizing the impact of EarthTec QZ on *B. tentaculata* while minimizing its effects on native species.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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