Relative Toxicity of Nickel, Potassium, and Chloride to Endemic Freshwater Gastropods in the Cahaba River Basin

by

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Abstract

Alabama is host to unprecedented freshwater gastropod endemism and increasing habitat change through urbanization and rural farmland development. Many freshwater gastropods are restricted to single catchments and are thus vulnerable to the environmental pollution associated with human activities. Previous studies have demonstrated the toxic capacity of nickel, potassium, and chloride (i.e., common urban and agricultural toxicants) to species present in Alabama. The goal of this thesis research is to further characterize the relative toxicity among three chemicals and three species/groups endemic to the Cahaba River in the Mobile River basin. We collected juvenile and adult prosobranch gastropods (*Somatogyrus georgianus*, *Elimia cahawbensis*, and *Elimia* spp.) from the Cahaba River and its tributary, Sixmile Creek, and conducted 18 96-hour acute toxicity tests split evenly among the three toxicants. The snails collected were approximately 60-120 days old based on knowledge of spawning seasons, although we used size as a proxy for age during field collection. We found nickel to be the most toxic pollutant tested, followed by potassium, and chloride. Among juvenile snails, *S. georgianus* was the most sensitive species/group to nickel and potassium, whereas *Elimia* spp. was the most sensitive species/group to chloride. The relative trends across species/groups changed for adult snails, although the relative sensitivity among the toxicants remained the same. This thesis research confirms the toxic capacities for nickel, potassium, and chloride and suggests novel trends in the effects of age on the toxicity of heavy metals to *Elimia* snails. Additionally, through

comparisons with published literature, this thesis reinforces the use of hardness-based Water Quality Criteria for conservation and management.

Artificial Intelligence (AI) Use Disclosure

In the preparation of this thesis, the following Artificial Intelligence (AI) tools were used: Google Scholar Labs and ChatGPT. These tools were used primarily to assist with the search for relevant scholarly articles and to edit code in R when developing figures. The author acknowledges full responsibility for the intellectual content of this work and has ensured that all AI-assisted sections have been reviewed and revised for accuracy and appropriate academic style. All AI-generated content was reviewed and validated for relevance, appropriateness, and accuracy before incorporation into the final document to maintain scholarly integrity of this research.

Digital Accessibility Use Disclosure Statement

In the preparation of this thesis, the following digital accessibility tools were used to ensure this document complies with federal requirements: Microsoft Word Accessibility Assistant. The author acknowledges full responsibility for the intellectual content of this work and has made a good faith effort to comply with digital accessibility requirements in publishing, wherein the nature of the content does not significantly change in order to do so. Furthermore, all content has been reviewed and revised to meet these requirements prior to final publication.

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Chapter 1: Literature Review and Research Objectives

Human activity and ecosystem compromise

Both industrial development and economic development have increased pressure on the environment. The increased demand for resources necessary to support continuous growth of the human population has led to increased exploitation of natural ecosystems. There are a myriad of examples of human-landscape modifications including wetland drainage for agriculture (Elo et al., 2015; Kitson et al., 2020), the clear-cutting of forest for urban developments (Keenan & Kimmins, 1993), river channelization to reduce flooding (Franklin et al., 2009) and promote transport of goods (Brooker, 1985), and dam construction for energy and drinking water (Dillon & Fishman, 2019; Bohada-Murillo et al., 2021). Together, these practices have improved food/water security and human welfare (Dillon & Fishman, 2019). However, anthropogenic environmental change applies new selection pressures on the inhabiting biota (Hua et al., 2024), thereby reshaping the community structure (Franklin et al., 2009; Elo et al., 2015; Newbold et al., 2015; Cooper et al., 2017; Turgeon et al., 2019) and permanently altering the ecoservices provided by the altered land. For example, Elo et al. (2015) and Szarek-Gwizada et al. (2023) documented that drained peatlands resulted in a decrease in insect biodiversity and Li et al. (2022) documented an increase in habitat fragmentation affecting wetland specialist species. Furthermore, Kitson and Bell (2020) and Szajdak et al. (2020) detected increases in CO_2 emissions due to the heightened rate of decomposition of exposed organic material that was once covered by water. Channelizing streams and reducing flood events decreases habitat availability and may lead to a degradation of surrounding wetland habitat (Franklin et al., 2009) and homogenization of inchannel habitat (Mollenhauer et al. 2022). Dam construction increases habitat fragmentation (Cooper et al., 2017) which disrupts freshwater mussel reproduction (Eads et al., 2015), disrupt spawning by fishes (Collins, 1976; Poff et al., 1997; Bunn & Arthington, 2002), and alter assemblage structure (Cooper et al., 2017; Turgeon et al., 2019).

Some species that have developed hyper-specific adaptations or strategies specific to a narrow range of habitat and climate conditions may be more susceptible to land-use changes than more generalist species (Newbold et al., 2018; Manes et al., 2021; Wurz et al., 2022; Chaves et al., 2025). These habitat specialists tend to be endemic to specific areas and have low dispersal capabilities, restricted geographic range often to single river catchments, and specialized niches (Ponder & Colgan, 2002). Freshwater organisms are particularly vulnerable to land-use change because of their isolation and reliance on hydrological connectivity (Fagan, 2002; Perkin & Gido, 2012). Therefore, restricted dispersal capacity of these endemic organisms limits their ability to recolonize isolated or degraded fragment populations (Thomas, 2000). Therefore, fragmentation and degradation of these habitats may favor disturbance-tolerant generalist species and select against ecological specialists (Newbold et al., 2018; Ramiadantsoa et al., 2018). Mykrä & Heino (2017) and Mykrä et al. (2025) demonstrated how freshwater stream macroinvertebrate assemblages shifted in favor of more tolerant species when exposed to anthropogenic land use change.

Pollutants, sources, and toxicity

Pollutants enter water systems through both non-point (e.g., agricultural and urbanized runoff) and point sources (e.g., wastewater effluent) linked to human activities (Brown & Peake, 2006; Kolahchi & Jalali, 2007; Shajib et al., 2019; Rey-Romero et al., 2022; Liu et al., 2023; Oladimeji et al., 2024; Sanusi et al., 2025). Pollutants, such as potassium, chloride, nitrogen, and phosphorus are considered as non-point source pollutants and come from fertilizer use and agricultural runoff (Carpenter et al.,1998). Other pollutants, such as heavy metals, pharmaceuticals and personal care products are known as point-source pollutants and come from industrial activities (e.g., metal smelting, mining, processing) and wastewater treatment and industrial effluents (Förster, 1996; Paul & Meyer, 2001; Shajib et al., 2019).

Industrial and municipal treated effluent, especially from metal smelting and processing facilities, can release copper, nickel, potassium, cadmium, zinc, lead, and chromium into the environment (Karvelas et al., 2003; Tytła, 2019; Oladimeji et al., 2024). In addition, building construction, highway infrastructure, and vehicle usage have been reported releasing metals into the environment (Brown & Peake, 2006; Shajib et al., 2019). For vehicles in particular, metals can be released through brake-wear, tire abrasion, and fuel combustion (Hall, 1972; Adamiec et al., 2016; Peikertova & Filip, 2016). Depending on the climate, potassium-based de-icers may also be used in urbanized areas in the winter months (Verkuil, 2024). Other common pathways for non-point source pollutants to enter waterways include potassium-based fertilizers that can wash into streams and aquatic environment (Kolahchi & Jalali, 2007; Rey-Romero et al., 2022). Though potassium is considered less toxic because it is an essential mineral, widespread use in the fertilizers does suggest potential for large influxes after storm events (Sandén et al., 1997). Importantly, metal exposure often occurs alongside other stressors such as altered flow regimes, increased temperatures (Poot et al., 2007), and additional other contaminants that may interfere with or amplify sensitivity (Cheng et al., 2025).

Many of these pollutants, especially metals, can accumulate in rivers and streambeds and become bioavailable to benthic organisms (Reynoldson, 1987; Hoang et al., 2008; Sharafi & Salehi, 2025). Metals are non-biodegradable and can remain bioavailable for decades (Ali et al., 2019) until remediation and/or cleanup actions occur.

Once metals enter aquatic ecosystems, they become consumable by fishes and aquatic invertebrates, which can lead to potentially bioaccumulation through consumption and can cause toxicity to organisms (Aoshima, 2017; Rehman, 2018). Humans at the top trophic level of the ecosystem can be exposed to metals via consumption of metal contaminated diet. According to Aoshima (2017), cadmium was accumulated in humans through consumption of aquatic vegetation polluted with cadmium that resulted in “Itai-Itai” disease in Japan.

The toxicity of metals to aquatic organisms varies widely as a function of species’ traits, exposure pathways, and environmental chemistry. Invertebrates and other ectotherms, especially filter feeders (e.g., some species of mollusk), are preferentially chosen for metal toxicity studies because of their sensitivity and likelihood of exposure (Naimo, 1995; Malaj et al., 2012). Sensitivity of aquatic organisms depends on their capacity to accumulate metals, and gastropods and other benthic freshwater invertebrates are particularly vulnerable to metal exposure. Their association with the sediment results in a two-pathway exposure through the water passing the gills and sediments they physically contact and ingest (Hoang et al., 2008; Besser et al., 2015; Waykar & Petare, 2016). Also, extended exposure time increases the sensitivity of organisms (Kenaga, 1982). In addition, metal bioavailability and toxicity are also influenced by water quality. Acidic water produces high fractions of ionic metals believed to be more bioavailable to aquatic organisms (Paquin et al., 2002; Hong et al., 2010; Wang et al., 2014). Meanwhile, calcium and magnesium (i.e., indicators of hardness) can inhibit metal ions at the biotic ligand and decrease metal uptake and therefore limiting the bioavailability (Paquin et al., 2002; Li et al., 2009).Dissolved organic carbon can complex with metals to form a non-bioavailable metal species, thereby decreasing metal bioavailability and toxicity (Heijerick et al., 2003).

Current toxicity data are often structured around model test species such as *Daphnia magna*, *Pimephales promelas*, *Danio rerio, Pomacea paludosa,* and various mussels in the genus *Mytilus* (Eaton, 1973; Alsop & Wood, 2011; Nadella et al., 2009; Okamoto et al., 2015; Hoang & Tong, 2015). Model organisms are chosen due to their ease to culture or collect and their sensitivity to pollutants (United States Environmental Protection Agency, 1994; Segner & Baumann, 2016). This is an important first step for determining the relative toxicity of pollutants. However, local species responses do not typically mirror those of model species, and there are potential conservation concerns that using model species mischaracterizes the risk of exposure to endemic species (Rubach et al., 2011; Ivan et al., 2024; Baia et al., 2025). To create a more robust understanding of endemic species' responses to toxicants, it is important to evaluate their sensitivities directly. However, a major limitation to understanding community toxicology is a poor characterization of toxicity on endemic species (Reichelt-Brushett et al., 2023).

Study Site and organisms

Alabama is one of the most biodiverse parts of North America and has undergone profound biodiversity loss. East of the Mississippi River, Alabama has the highest freshwater biodiversity (Duncan & Wilson, 2013). In fact, Alabama holds a disproportionately large percentage of freshwater fishes, mollusks, and reptiles (Lydeard & Mayden, 1995) Nearly half (43%) of the gill-breathing snails present in North America are found in Alabama and 28% are endemic (Lydeard & Mayden, 1995; Johnson, 2013). Endemic organisms are at higher risk of extinction (Thomas, 2000), which threatens the biodiversity of Alabama's river systems. Freshwater snails, like other freshwater mollusks, are experiencing rapid population declines and increased extinction and extirpation rates (Johnson et al., 2013; Régnier et al., 2009). Johnson et al. (2013) indicated that only 22% (of 703 species in the U.S. and Canada) of gastropod species are thought to have stable populations. Whelan et al., (2012) attributed the Mobile River Basin mollusk extinctions totaling one-third of documented global freshwater mollusk extinctions.

The freshwater snails in the Cahaba River, Alabama are exposed to a myriad of anthropogenic threats as the river traverses both rural and urban areas that introduce a variety of different pollutants. Urban sprawl in central Alabama increased the need for wastewater infrastructure, and although there are regulations on effluent waters, heavy metals and other toxicants can still be released (Karvelas et al., 2003; Tytła, 2019; Oladimeji et al., 2024). Urban growth also worsens non-point source pollution from increased impervious substrate (Paul & Meyer, 2001). Runoff from pavement/roads (Paul & Meyer, 2001), roofs (Förster, 1996), and degraded forests carry additional heavy metals that degrade water quality and disrupt ecosystem functions. Rural areas, meanwhile, contribute high nutrient loads via fertilizer runoff (Kolahchi & Jalali, 2007; Rey-Romero et al., 2022). Potassium-based fertilizers, most commonly potassium chloride (KCl), introduce potassium and chloride ions that can alter water chemistry, lower pH, and exert toxic effects on aquatic organisms (Gibson et al, 2018). Although potassium itself is not well understood, the associated chlorine may harm aquatic snails, making both urban and agricultural pollution significant concerns for Alabama's aquatic mollusks.

Research objectives

This thesis research aims to improve our understanding of the effects of toxicants commonly associated with land use change on narrow-range endemic species of Alabama gastropods. The specific objectives are to 1) determine the relative sensitivities – via lethal endpoints – of two species and one species group of narrow range endemic snails to three toxicants commonly found in Alabama rivers, 2) determine the effects of relative age (i.e., size) on the sensitivities of the snails to further our understanding of life-history toxicity for management and conservation purposes, and 3) determine the bioaccumulation of the metal toxicants in the tissue of the snails. Ultimately, these data will provide an important framework for adding novel species into toxicological literature for consideration in Alabama Water Quality Criteria, providing local risk assessments for other Cahaba River Basin aquatic taxa.

Chapter 2: Methods

Snail collection and laboratory acclimation

We sampled three snail species/groups from two sites in the Cahaba River in central Alabama. On May 31st, we collected juvenile *Somatogyrus georgianus* and juvenile *Elimia* spp. from the lower Little Cahaba River (33.169 ^{o}N, 87.022^{o} W), and we collected juvenile *Elimia cahawbensis* from Sixmile Creek (33.008^{o} N, 87.004^{o} W) in Bibb County, Alabama. Collections took place in late spring, 2024, approximately two months post egg laying and hatching. Snails were collected from rocks located on the stream bed and transferred to small containers after being identified to species. All species that were not part of the experiment were released back into the stream. The majority of *Elimia* spp. collected were likely *Elimia clara*, but diagnostic characters are limited with juvenile pleurocerid gastropods, so definitive identification was not possible. Additionally, taxonomic reviews for Cahaba Basin Elimia are currently under further review (Whelan et al., 2022). During collection in the lower Little Cahaba River, we separated the juvenile *Elimia* spp. from other pleurocerids. *Somatogyrus georgianus* were determined by their relatively small size and behavior (i.e., active antennal movement and rapid righting behavior when inverted). Finally, all snails present at the

Sixmile Creek sample site were *Elimia cahawbensis*. Our collections totaled 630 *Elimia* spp., 630 *E. cahawbensis,* and 660 *S. georgianus* at each collection interval.

Adult snails were collected at the same sites and using the same methods; however, we selected larger snails and the collection timing differed by species/group. We collected snails over two trips to the Cahaba River. *Somatogyrus georgianus* and *Elimia* spp. were collected from the lower Little Cahaba River on September 12th, 2024, and the *Elimia cahawbensis* were collected from the Sixmile Creek on August 2nd, 2024. Initially, we planned for the collection times to be approximately four months post-hatch. However, scheduling complications pushed back the collections for *S. georgianus* and *Elimia* spp. by another month. We used size as a predictor of age for field collection purposes; therefore, we considered juvenile *Elimia* were < 1 cm in shell length (anterior-posterior axis), and juvenile *Somatogyrus* < 1 mm in total length. For the adults, we looked for *Elimia* and *Somatogyrus* that were > 2 cm and > 2 mm in shell length, respectively. Snails were transported in chilled and aerated containers to the laboratory at Auburn University where they were acclimated to laboratory conditions.

In the laboratory, we acclimated the snails to artificial moderately hard water (MHW) at 25°C and a photoperiod of 16h light and 8h dark for a minimum of two days before conducting experiments (Leverett and Thain, 2013; Barrick, 2018). Our MHW was prepared by dissolving salts in de-ionized water (4 mg/L of KCl, 60 mg/L $CaSO_4 \bullet 2H_2O$, 60 mg/L $MgSO_4$, and 96 mg/L $NaHCO_3$; U.S. EPA, 2002) to mimic the natural water quality of the Cahaba River (Table B1). The temperature and water quality of the MHW were 25.4°C, 78 mg/L as $CaCO_3$ (hardness), 88 mg/L as $CaCO_3$ (alkalinity), 7.4 (pH), and 0.1 ppt (salinity). Snails were provided with biofilm growth on rocks that we collected from the respective sample sites. Continuous aeration was used to maintain oxygen concentrations above 6 mg/L during the acclimation period.

We took average weights and operculum lengths of at least 30 snails of each species/group to create a baseline prior to experimentation (Table B2). Snails were weighed on a Toledo Balance (VWR International Ltd, Suwanee, Georgia, USA) with 0.01 mg accuracy. However, because of the low weight (< 0.01 mg) of *S. georgianus*, we measured in three batches of ten snails to estimate average individual weight. Additionally, we measured the width of the apertural opening, shell length, and shell height (widest portion of the shell perpendicular to the shell length) for each snail included in the subsamples. For the *Elimia* snails, we used an electronic caliper (Traceable 62379-531) with an error of 0.03 mm to conduct our measurements, whereas we measured the *Somatogyrus* snails using a LAXCO dissection microscope (LAXCO Inc, Mill Creek, WA, USA). Size and weight data were recorded to document morphological characteristics of these species and to provide wet-weight conversions for accumulation when dry-weights were unattainable (Table B2).

Toxicity Tests

Following standard U.S. EPA methodology (U.S. EPA, 2002), we used 96-hour static renewal acute toxicity tests to conduct experiments on the relative sensitivities of the three species/groups to three chemicals: nickel (Ni), potassium (K), and chloride (Cl). Each experiment used six chemical concentrations and a control. We replicated our trials three times, including ten snails per replicate (n = 21 test chambers, 210 snails per trial). Toxicity experiments were conducted in 800 mL and 250 mL polypropylene beakers, containing 750 mL and 200 mL test water (MHW), respectively. Laboratory conditions during testing were 25 ± 1 ^{o}C, with a photoperiod of 16 hours light and 8 hours dark. We placed the *Elimia* snails in the 800 mL beakers and the *Somatogyrus* in the 250 mL beakers because of the size differences. Every 24 hours after the start of an experiment, we collected mortality data and removed dead snails. Dead snails found during mortality data collection were removed from test containers, put in labelled polypropylene tubes, and frozen at -20°C. Mortality was determined through gentle mechanical stimulation of the snail tissue and through a 15-minute observation in control water. Snails were considered dead if there was no response (i.e., no opercular movement) to either of these tests. After 48 hours, we replaced the test water in each beaker with freshly prepared test solution and took samples to verify toxicant concentrations. We did not feed the snails during the experiments to reduce the possibility of contamination.

We monitored water quality parameters throughout each experiment to ensure consistent conditions. Concentrations of water hardness and alkalinity were measured at the beginning and end of each experiment, whereas temperature, salinity, dissolved oxygen (DO), and pH were measured daily. We used a YSI probe (Xylem, Yellow Spring, Ohio, USA) to measure temperature, salinity, and dissolved oxygen, and a Toledo pH meter (Mettler-Toledo LLC, Columbus, Ohio, USA) to measure pH. Hardness and alkalinity were determined through titration with 0.01M of ethylenediamine tetraacetic acetate (EDTA) and 0.2N H_2SO_4, respectively. In Cl trials, we could not determine hardness from titration because of elevated Ca concentrations from the use of calcium chloride, and instead we calculated the hardness from the concentrations of Ca and Mg detected in the water samples analyzed via ICP-MS

Test concentrations were prepared from stock solutions of nickel chloride, potassium sulfate, and calcium chloride. Our goal was to isolate the effects of both potassium and chloride, so we avoided using the single chemical, potassium chloride (KCl), and opted for lesser-used salts. The Ni, K, and Cl concentrations for both juvenile and adult experiments ranged from 0 – 16 mg/L Ni, 0 – 2000 mg/L K, and 0 – 8000 mg/L Cl. The concentrations used in adult experiments were typically twice as concentrated as those used in juvenile experiments.

We collected water samples from the test chambers to verify toxicant concentrations and collect data on mineral elements (Ca, Mg, Na, K) and anion (Cl) concentrations. Samples were collected from the water before and after running the experiment and then filtered through a syringe and 0.45 μm filter. Prior to analysis, we preserved samples in a refrigerator at 5°C. Samples designated for metal analysis had one drop of nitric acid added to keep the dissolved metal stable. We then analyzed cations (Ni, K, Ca, Mg, and Na) with an inductively coupled plasma mass spectrometer (ICP-MS), whereas the anion, Cl, was analyzed via an ion chromatographer.

Digestion and Bioaccumulation

We measured tissue accumulation of Ni and K in snails after toxicity trials to determine bioaccumulation concentrations. All individuals (i.e., live and dead) were removed from the test chambers and rinsed three times with Milli-Q water to remove residual test chemicals from the shell surface. Snails were sorted by treatment replicate, placed into 15-mL polypropylene tubes, and stored at −20 °C until later analysis. On the day of digestion, samples were thawed at laboratory temperature (~25 °C) and weighed by replicate to obtain wet mass. For *Elimia* spp. and *E. cahawbensis*, we separated the soft tissue and shell prior to weighing and digestion, whereas *S. georgianus* individuals were weighed whole because their small size prevented accurate dissection without contamination. Following wet weight measurements, samples were dried at 80 °C in a convection oven (VWR International Ltd., Suwanee, Georgia, USA) for 24 h, then cooled to laboratory temperature in a desiccator for an additional 24 h before being reweighed to determine dry mass. Due to the small size of the juvenile snails, dry weights could not be accurately obtained as measurement error of the balance regularly exceeded the dry weight of the sample, providing negative values. Therefore, wet weights were used for all juvenile accumulation concentrations, whereas dry weights were used for adult accumulation concentrations. Dry weight data were used for adult bioaccumulation as this is standard practice (Barrick et al., 2024) and is important for future bioaccumulation studies.

We digested the snail tissue samples with HNO_3 following U.S. EPA method 3050B (U.S. EPA, 1996b) with modifications by Hoang et al. (2011) and Barrick et al. (2024). Briefly, the standard method was modified to digest smaller quantities and to prioritize the digestion of snails instead of sediments. We heated the samples to 95°C and added 50% HNO_3 to submerge and dissolve the shell. When the shells were fully dissolved, we reduced the temperature to 85°C and added 30% hydrogen peroxide in regular 15-minute intervals until the tissue was fully dissolved. Quality control (QC) samples (National Institute of Standards and Technology Trace Elements in Water

1643f) were included with the snail digestion. We analyzed the digested samples using ICP-MS (Model NexION 2000) to determine the total concentrations of Ni and K in the tissue and shells of the *Elimia* snails and the whole body of *S. georgianus*.

Data Analyses

We calculated lethal concentration (LC) and lethal accumulation (LA) values from mortality data and CETIS software (Comprehensive Environmental Toxicology Information System™; Tidepool Scientific LLC., McKinleyville, California, USA). Our LC and LA values were calculated using the logit method that is integrated into the CETIS software. Mortality data and measured dissolved concentrations of Ni, K, and Cl were used to calculate lethal concentrations that caused 10% (LC10), 15% (LC15), 25% (LC25), and 50% (LC50) mortality in our sample populations. In contrast, we used the tissue Ni accumulation concentrations in place of the dissolved Ni concentration to calculate LA values for Ni. The LA calculations were not possible for K or Cl; potassium tissue concentrations were insufficient for calculations, and Cl does not accumulate in tissue. We also ran a Pearson correlation analysis in Excel between the LC and LA values across all species/groups to evaluate any differences in the trends.

We used Program R (version 4.4.1) and R studio (version 2024.09.0 +375) to build Michaelis-Menten models and calculate associated coefficients. Following Barrick et al. (2024), we used an altered Michaelis-Menten kinetic model to compare the accumulation of Ni in the snail to the concentration of Ni in the test water. Michaelis & Menten (1913) originally designed the formula for the model to be used for enzymatic rates of reaction. Part of their formula is the Michaelis-Menten constant (K_M) that denotes the time at which half of the product concentration has been achieved and V_{MAX} denotes the maximum concentration of product possible. Since we used their formula to compare exposure concentrations to accumulation and saturation in the tissue, we do not report our Michaelis-Menten constant (K_M) as a time, but rather as an ambient toxicant concentration at which an organism has achieved 50% saturation within the tissue. Additionally, in our modified formula, the V_{MAX} is the mean saturation concentration (i.e., the maximum concentration possible in the organism) according to our model. The coefficients (K_M and V_{MAX}) are calculated using the "drm" function in the "drc" package (Ritz et al., 2015) available in R. We used the "ggsci", "ggplot2", and "patchwork" packages for the graphical representation of our data.

We developed simple linear models in R to evaluate the effects of exposure time (24, 48, 72, and 96 h) on LC50 for all species/groups and toxicants. We developed 18 linear models using the basic "lm" function in R for each species/group, toxicant, and age class. By using these linear models, we were able to statistically determine if time significantly affected the toxicity of each toxicant to each species/group. Additionally, by running an ANCOVA, we compared the resulting slopes from the models to determine if any were statistically different from one another so we could make claims on site-dependent, species-dependent, or family-dependent effects. Finally, all linear models were graphically represented using the "ggplot2" and "patchwork" packages in R.

Because data on mechanisms of K toxicity in gastropods are poorly documented, we analyzed cation (Ca, Mg, Na, and K) concentrations in both the test water and the tissue of the snails to identify potential trends in ion transport. In Excel, we ran a Pearson correlation analysis of the changes in concentrations over time for K in the water (K_W), K in the tissue (K_t), Ca in the tissue (Ca_t), Mg in the tissue (Mg_t), and Na in the tissue (Na_t). The goal was to detect ion concentration shifts that may indicate a change in ion-channel activity (e.g., sodium-potassium pump inhibition).

Chapter 3: Results

Water quality

The tested water quality parameters remained constant for all Ni trials but varied in terms of salinity and hardness for the K and Cl trials. The use of potassium sulfate at concentrations approaching 2 g/L increased the salinity to 2.2 g/L. Similarly, salinity increased in Cl trials from calcium chloride concentrations approaching 10 g/L. The salinity increased proportionally to the test concentrations in both age classes of K and Cl trials (Tables A1 & A2). Hardness was consistent for the K trials; however, it was orders of magnitude greater for the Cl trials (Tables A1 & A2).

The water samples collected for confirming nominal concentrations showed Ni and Cl concentrations were accurate, but the K concentrations had higher variation. Measured dissolved Ni concentrations were, on average, within approximately 15% of the nominal concentrations (Tables A3 & A4). Juvenile Ni experiments were more accurate than the adult experiments. On average, measured Cl concentrations were within 16% of nominal concentrations (Tables A5 & A6) and measured dissolved K concentrations were within 40% of nominal concentrations (Tables A7 & A8). Adult experiments with K and Cl were more accurate than juvenile experiments (Tables A5-

A8). The variation in K concentrations was not expected to affect data analysis or interpretation since all calculations used measured rather than nominal concentrations.

Juvenile lethal concentrations

Juvenile LC values tended to decrease with exposure time, with both the magnitude and rate of that decrease varying with species/group and toxicant (Figure 1). The exception to this trend was *S. georgianus* exposed to potassium (Table 1). There was a single mass mortality event after 24 h; all remaining *S. georgianus* survived to the end of the experiment. This caused the 48-h, 72-h, and 96-h LC values to be the same. The LC50s decreased following a linear function (Table 2; Figure 1). The slope of the relationship between LC50 and time was steepest for *E. cahawbensis* and flattest for *S. georgianus* exposed to Ni, whereas slope was steepest for *Elimia* spp. and flattest for *S. georgianus* when exposed to Cl. The K data for both *Elimia* species/groups consisted of two LC50 data points so comparisons may be invalid; however, the slope was steepest for *E. cahawbensis* and flattest for *S. georgianus*.

We could not calculate some LC values for some species/groups (Table 1) because of insufficient mortality data. LC calculations require near-complete mortality at the highest concentrations and statistically insignificant mortality at the lowest concentrations relative to the control. We observed snails lasting multiple days in the highest concentrations of *Elimia* spp. exposed to K that prevented calculation of LC values.

Resulting juvenile LC values indicated that sensitivity among species/groups varied by toxicant (Table 1, Figure 1), and ANCOVA supported the differences in LC50 trends over time (Table 2). Generally, *S. georgianus* was the most sensitive species (lowest LC values), followed by *Elimia* spp. and *Elimia cahawbensis*. The 96-h LC50 for *Elimia cahawbensis* exposed to Ni was 1.6 times higher than the 96-h LC50 of *Elimia* spp. and five times higher than the 96-h LC50 of *S. georgianus* exposed to Ni. *Elimia* spp. and *E. cahawbensis* had similar 96-h LC50s in response to K, and these were approximately 3.5 times greater than the 96-h LC50 of *S. georgianus* exposed to K. Chloride exposure showed a reverse trend relative to potassium. *Elimia* spp. and *E. cahawbensis* were the most sensitive species and their 96-h LC50s were approximately 3.8 times lower than the 96-h LC50 of *S. georgianus* exposed to Cl. ANCOVA from the juvenile LC50 linear models (Table 2) for Ni approached but did not achieve significance between *S. georgianus* and *E. cahawbensis* ($t = -3.497$, $df = 4$, $p = 0.053$) and no significant difference was detected between *S. georgianus* and *Elimia* spp. ($t = -0.807$, $df = 4$, $p = 0.719$) or *E. cahawbensis* and *Elimia* spp. ($t = -2.251$, $df = 4$, $p = 0.175$). ANCOVA for K and Cl could not be meaningfully interpreted as they compared linear regressions with perfect fits ($R^2 = 1$).

Adult lethal concentrations

Adult LC50s, like the juvenile LC50s, decreased as exposure time increased (Table 4); the rate and magnitude of decrease varied by toxicant and species/group (Table 3; Figure 2), with a single notable exception in *S. georgianus* exposed to K. The slope of the relationship between time and LC50 was steepest for *E. cahawbensis* and flattest for S. georgianus in Ni trials. For K trials where slopes could be meaningfully calculated, the slope was steepest for *E. cahawbensis* and flattest for *Elimia* spp. The slope was steepest for *E. cahawbensis* and flattest for *Elimia* spp. in the Cl trials. The slopes for *Elimia* spp. were similar to those of *S. georgianus* in Ni and Cl exposures but were steeper than *S. georgianus* for K exposures. LC50 data were limited for *E. cahawbensis* exposures to K and *Elimia* spp. exposures to Cl; slopes were generated from only two data points and resulting regression lines had limited meaningful interpretation. The exception to the decrease in LC50 over time was *S. georgianus* exposed to K. *Somatogyrus georgianus* experienced a single mass mortality event that killed all snails in the two highest K test concentrations after 24 hours. No *S. georgianus* died after 24 hours, resulting in a slope of zero.

Adult LC relative sensitivities varied with exposure time (Table 3, Figure 2) and LC50 trends over time were supported by ANCOVA between the adult linear models (Table 4). *E. cahawbensis* was the least sensitive for all exposure times to Ni, whereas *Elimia* spp. and *S. georgianus* showed similar sensitivities for all data points available. Potassium exposure revealed that *E. cahawbensis* remained the least sensitive species, yet the relative sensitivities changed for *Elimia* spp. and *S. georgianus* over time. At 48 hours, *Elimia* spp. was the least sensitive group. After 72 hours of exposure, *S. georgianus* was the most sensitive species. Chloride exposure showed that relative sensitivity shifted with exposure time. *Elimia cahawbensis* was the least sensitive species/group exposed to Cl at 48 hours but became the most sensitive species after 72 hours. As observed with Ni exposure, *Elimia* spp. and *S. georgianus* exposed to Cl showed similar sensitivities for all data points available. ANCOVA from the adult LC50 linear models (Table 4) for Ni showed significant differences between *S. georgianus* and *E. cahawbensis* ($t = -6.388$, $df = 4$, $p = 0.007$) and between *E. cahawbensis* and *Elimia* spp. ($t = -5.080$, $df = 4$, $p = 0.015$) but not between *S. georgianus* and *Elimia* spp. ($t = -0.316$, $df = 4$, $p = 0.947$). ANCOVA for K showed no significant differences between *S. georgianus* and *Elimia* spp. ($t = -2.706$, $df = 3$, $p = 0.142$). ANCOVA for Cl showed no significant differences between *S. georgianus* and *E. cahawbensis* ($t = -1.978$, $df = 3$, $p = 0.264$). Remaining pairwise slope comparisons for K (i.e., *E. cahawbensis*) and Cl (i.e., *Elimia* spp.) could not be meaningfully interpreted as they compared linear regressions with perfect fits ($R^2 = 1$).

Adult LC values were generally higher than juvenile LC values across all three species/groups and toxicants, with two exceptions observed in *Elimia* spp. Adult 96-h LC50s were higher than juvenile values for both *S. georgianus* (1.4x, 2.5x, and 1.3x for Ni, K, and Cl, respectively) and *E. cahawbensis* (1.5x, 1.3x, and 1.7x for Ni, K, and Cl, respectively). Adult *Elimia* spp. 96-h LC50s followed the same pattern for Cl (5.5x higher); however, the values were 1.5x and 4.8x lower for Ni and K, respectively.

Juvenile lethal accumulation and bioaccumulation

Juvenile lethal accumulation concentrations were calculated for Ni only and varied by species/group across effect percentages (Table 5). Potassium data were insufficient for accumulation calculations due to limited mortality and poor organism condition from tissue disintegration at the time of collection, and Cl does not accumulate in tissue. Though *S. georgianus* had the lowest LA values and *E. cahawbensis* had the highest values, the relative trends of lethal accumulation values varied across effect percentages. The LA10, LA15, LA25, and LA50 of *Elimia* spp. were 12.6, 9.4, 3.9, and 3.4 times greater, respectively, than those of *S. georgianus.* The LA10, LA15, LA25, and LA50 of *E. cahawbensis* were 40.3, 31.4, 15.3, and 17.7 times greater than those of *S. georgianus*, respectively, and 3.2, 3.4, 3.9, and 5.3 times greater than those of *Elimia* spp., respectively. A Pearson correlation analysis between LC and LA values showed a strong positive correlation across all species/groups and LC/LA effect percentages (LC50:LA50 = 0.96, LC25:LA25 = 0.95, LC15:LA15 = 0.96; Table 6).

Nickel accumulation followed the saturation pattern described by the modified Michaelis-Menten kinetic model (Figure 3). *Elimia cahawbensis* had the highest potential for accumulation (C_{MAX} = 0.203 $\pm$ 0.016 mg/g ww, $p < 0.001$) and reached 50% saturation at the lowest ambient Ni concentration (K_M = 433.89 $\pm$ 90.67 μg/L Ni). *Elimia* spp. had a lower maximum concentration (C_{MAX} = 0.041 $\pm$ 0.008 mg/g ww, $p < 0.001$) but reached 50% saturation at a higher ambient Ni concentration (K_M = 517.28 $\pm$ 207.02 μg/L Ni) compared to *E. cahawbensis*. *Somatogyrus georgianus* had the lowest accumulation potential (C_{MAX} = 0.012 $\pm$ 0.023 mg/g ww), though it was not significant (p = 0.609). The K_M of *S. georgianus* could not be reliably estimated (9467 μg/L Ni) as the standard error was incalculable, and this value was outside the tested concentrations of Ni.

Cation accumulation (Ca, Mg, Na, and K) varied across species/groups in response to increasing ambient K concentrations (Table 7). Potassium in the body of *Somatogyrus georgianus* decreased as ambient K concentrations increased, whereas K accumulation in both *Elimia* species/groups increased with ambient K. The correlation between ambient and accumulated K was strong for *Elimia* spp. only (r = 0.91). Magnesium and sodium were negatively correlated with ambient K concentrations across all species/groups, with the strongest correlation observed for Mg in *S.*

georgianus (r = -0.67) and Na in *E. cahawbensis* (r = -0.54). Calcium was negatively correlated with ambient K concentrations for all species, but the strongest correlation was in *E. cahawbensis* (r = -0.67).

Adult lethal accumulation and bioaccumulation

We calculated the adult LA values for Ni only and found that they varied by species across effect percentages (Table 8), though high Ca concentrations and poor organism tissue condition (i.e., disintegrating tissue) precluded analysis of the adult *Elimia* spp. and K samples, respectively. The LA10, LA15, LA25, and LA50 of *E. cahawbensis* were 3.5x, 4.0x, 4.9x and 9.3x higher, respectively, than those of *S. georgianus*. No comparisons could be made for *Elimia* spp. as the Ca concentrations present in the samples were too high to analyze in the ICP-MS; dilution of samples to Ca concentrations low enough to analyze the samples would inhibit the analysis of Ni. As a result, LA values for *Elimia* spp. could not be calculated.

Both *S. georgianus* and *E. cahawbensis* followed the modified Michaelis-Menten kinetics for saturation (Figure 4). *Elimia cahawbensis* had the highest potential for Ni accumulation (C_{MAX} = 2148.7 $\pm$ 1137.9 mg/kg dw, p = 0.070) and K_M of 2966.4 ($\pm$ 4421.3, p = 0.508) μg/L Ni. *Somatogyrus georgianus* had the lowest potential for Ni accumulation (C_{MAX} = 183.6 $\pm$ 52.4 mg/kg dw, p = 0.002) and K_M of 440.7 ($\pm$ 399.06, p = 0.281) μg/L Ni. No parameter in the Michaelis-Menten model was statistically significant for *E. cahawbensis*, and only the C_{MAX} was significant for *S. georgianus*. The wide standard errors in both models suggest high uncertainty in the K_M estimates.

Direct comparisons of accumulation between age classes were generally not possible due to the difference in weights between age classes. Juvenile accumulation was reported as wet weight whereas the adult accumulation was reported as a dry weight. K_M values should be unaffected by this distinction, but the only comparable species was *E. cahawbensis* due to the lack of *Elimia* spp. data and unreliable K_M estimate for *S. georgianus*. Juvenile and adult *E. cahawbensis* reached 50% tissue saturation at approximately the same ambient Ni concentration (433.9 mg/L juvenile, 440.7 mg/L adult)

Chapter 4: Discussion and Conclusions

The results of the present study are novel for these Cahaba River snails, indicating that the toxicity of Ni, K, and Cl was species dependent and varied among species/groups and life stage. Juvenile snails were more sensitive than adult snails, except for the case of *Elimia* spp. Early life stages of freshwater gilled animals often have less developed defense mechanisms, organ systems, and detoxification capacities making them more vulnerable to toxicants (Mohammed, 2013; Cadmus et al., 2019). Mácová et al. (2008) reported that the LC50 values of zebrafish (*Danio rerio*) exposed to 2-phenoxyethanol increased consistently with the age of the organisms from embryo to juveniles. Hoang et al. (2004) found that nickel toxicity to fathead minnow decreased as a linear function of organism age. Stuhlbacher et al. (1993) demonstrated the tolerance of *Daphnia magna* to cadmium increasing with age. Similarly, Rogevich et al.

(2008) reported that old apple snails were less sensitive to copper than younger snails. However, we found *Elimia* spp. adults were more sensitive than the juveniles to both Ni and K, which has been found by others. Hoang & Klaine (2007) found that the most sensitive age for *D. magna* exposed to various metals was 96-hours post-hatch and McNulty et al., (1994) determined that *Atherinops affinis* of <1 week old were less sensitive than those ≥1 week old. Although we cannot explain this phenomena, one possibility is that we assumed that *Elimia* spp. was *Elimia clara*, but species-level identification was uncertain because of the morphological plasticity of pleurocerids in the Mobile River basin (Whelan et al., 2019). *Elimia* spp. was the largest group we used in the study, and currently the mechanisms leading to the increased adult sensitivity are unknown and warrant further investigation. Relative sensitivity was not constant across all three toxicants, indicating that the toxicity was influenced by both species-specific physiological characteristics and life-history strategies.

Nickel sensitivity and accumulation

Nickel sensitivity varied across species/groups and life stage. Among juvenile snails, *S. georgianus* was the most sensitive species, *Elimia* spp. was intermediate, and *E. cahawbensis* was the least sensitive. Smaller organisms are typically more sensitive to metal exposure (Mohammed, 2013; Cadmus et al., 2019). Similar patterns are seen in other freshwater snails and aquatic taxa. Smaller *Pomacea insularum*, a species of South American apple snail, are more sensitive to several heavy metals than larger members of the same species (Yap et al., 2023). Cadmus et al. (2019) showed that increasing size classes of various aquatic insects increased tolerance. This trend held for *E. cahawbensis* and *S. georgianus* because the LC50 values increased with size. However, *Elimia* spp. did not follow this pattern as the larger adult snails were more sensitive to Ni exposure than the smaller juvenile snails. These results indicate that size-related changes (and corresponding age-related changes) in nickel tolerance were species-dependent instead of universal across all taxa. The mechanism responsible is unknown, although it may be a consequence of changes in ion-transport, sequestration capabilities, metabolism, or a myriad of other physiological traits possibly associated with changes in age (The Task Group on Metal Interaction, 1978; Mohammed, 2013).

We found Ni accumulation did not scale proportionally to Ni exposure sensitivity. Although *E. cahawbensis* was the least sensitive species/group, it had the highest Ni accumulation. The LA values of *E. cahawbensis* were the highest among the LA values found for all three snail species. These results indicate that not all accumulated Ni was bioavailable or toxic to the snails. Under the assumptions of the biotic ligand model, metal toxicity to fish is related to the metal accumulation at the biotic ligand, specifically at the gill membrane (Paquin et al., 2002; Hoang et al., 2008). As freshwater gilled mollusks and fish have similar gill structures related to ion transport (i.e., sodium-potassium pumps), it is assumed that the principals of the biotic ligand model are comparable between the taxa (Hoang et al., 2008). Additionally, mollusks can bind metals to proteins (i.e., metallothionein; Langston et al., 1998) and calcium/magnesium phosphate granules (Marigómez et al., 2002), thereby reducing the bioavailability and toxicity. The relatively high tissue Ni burden in *E. cahawbensis* suggests that this species might have a higher potential to sequester Ni than *S. georgianus* or *Elimia* spp.

More research should be conducted to study the potential Ni sequestration in these snail species and to explain the differences in Ni accumulation and toxicity.

Differences found between our LC50 values and those reported in previous research characterizing Ni toxicity to these freshwater gastropods likely depend on the test conditions. Barrick et al. (2024) characterized the lethal responses of the same juvenile species used in the present study to Ni exposure, whereas Gibson et al. (2018) examined the sublethal responses of mollusks, including *S. georgianus*, to multiple toxicants (i.e., nickel, zinc, copper, and potassium). Therefore, there are no experiments present in all three studies for direct comparisons. However, we found that our 96-h LC50 values were higher for every juvenile species compared to a study by Barrick et al. (2024). Our organisms were exposed to moderately hard water (~88 mg/L $CaCO_3$) whereas Barrick et al. (2024) used soft water (~20 mg/L $CaCO_3$), which is uncommon for the Mobile River basin (State of Alabama Water Improvement Advisory Commission, 1949; Pitt, 2000; Barrick et al., 2024). Additionally, Barrick et al. (2024) used cultured organisms, whereas the present study used wild-caught snails. The use of wild-caught organisms hindered our ability to accurately assess age classes in our experiments, and as we have shown, age can affect sensitivity greatly. Therefore, the differences between previous experiments are likely an artifact of differences in design.

The current U.S. EPA nationally recommended freshwater Ni criteria were published in 1995 and set a criterion maximum concentration (CMC) of 0.43 mg/L Ni (adjusted to our water hardness of 88 mg/L; U.S. EPA, 1996a). This is also the CMC used for regulations in Alabama (Alabama Department of Environmental Management, 2017). This Ni CMC was derived from available Ni LC50 data dating back to 1995 that did not include toxicity data for freshwater gilled gastropods. All 96-hour Ni LC50 values calculated in this study were higher than the adjusted CMC. However, the LC25 of *S. georgianus* was 0.43 mg/L Ni and the LC15 was 0.35 mg/L Ni. The Water Quality Criteria are established based on the expectation that 90% of organisms exposed should not experience significant risk (U.S. EPA, 1996a). Therefore, incorporation of these results into a revised EPA or Alabama Department of Environmental Management (ADEM) Ni criterion would better protect these important gastropod species in the aquatic ecosystems.

Potassium sensitivity

Potassium produced strong species- and age-dependent responses that followed similar trends to the Ni exposures. Like we observed in Ni experiments, *E. cahawbensis* was generally the least sensitive species/group, whereas *S. georgianus* was more sensitive in comparison, especially with juvenile snail experiments. However, we observed the sensitivity of *Elimia* spp. increasing with age from juvenile LC values close to *E. cahawbensis*, the least sensitive species, to LC values lower than those of *S. georgianus*. These data suggest that K toxicity to these gastropods, like Ni toxicity, is not governed by solely body-size, even with snails within the same genus. However, further research is necessary to understand the mechanisms for the drastic changes in *Elimia* spp. sensitivity.

Our K results support literature suggesting the use of K as a molluscicide as our snails are highly sensitive compared to other previously researched mollusks. Wang et al. (2017) demonstrated that five freshwater mussel species experienced EC50 concentrations between 31 and 56 mg/L K. Juvenile *S, georgianus* and adult *Elimia* spp. were the only species/groups to experience LC50 values below these thresholds, but the distinction between EC50 and LC50 should be noted. Lethal concentrations are logically expected to be higher than effect concentrations as effects will occur before death. This indicates that our gastropods have the potential to be more sensitive to K than previously tested mollusk species. However, Gibson et al. (2018) determined that two Alabama gastropods, adult *S. georgianus* and juvenile *Leptoxis ampla,* and two mussel species, *Hamiota perovalis* and *Villosa* nebulosa, had 96-h EC50s of 7.29, >1.000, 11.94, and 15.97 mg/L K, respectively (Gibson et al., 2018). These data suggest *S. georgianus* in our study (96-h LC50 = 65.98 mg/L K, Table 3) may be more tolerant than those previously tested in the literature. Like Barrick et al. (2024), the disparity in our results could be explained by differences in water hardness (~44 vs ~88 mg/L $CaCO_3$) and organism source (i.e., cultured organisms; Gibson et al. 2018). More research would be beneficial to understand how water quality might influence the toxicity of K on endemic gastropods.

Potassium may be more damaging from ion regulation than other heavy metals (i.e., Ni) that are more directly toxic through accumulation at a biotic ligand (Paquin et al., 2002). Tissue K only increased strongly with ambient K in *Elimia* spp., whereas Na, Mg, and Ca generally declined across all species/groups as ambient K increased (Table 7). This pattern supports the idea that elevated K interfered with the normal cation regulation (Barron et al., 2015; Wang et al., 2017, Wang et al., 2023). Additionally, we observed rapid decomposition and bloating in dead snails exposed to K that also indicates osmotic imbalance. That interpretation is consistent with data on the mode of action for K (Wang et al., 2017).

Unlike Ni, the U.S. EPA and ADEM do not have established water quality criteria for K (Alabama Department of Environmental Management, 2017). Though current field concentrations were below acute lethal concentrations, the margin is relatively small compared to other toxicants. This highlights the necessity for further research as we have demonstrated the sensitivity of narrow range endemic gastropods to chemicals present in rivers and streams.

Chloride sensitivity

Chloride sensitivity followed a different pattern from Ni and K between age classes. With juvenile organisms, *S. georgianus* was the least sensitive species/group, whereas *E. cahawbensis* and *Elimia* spp. were more sensitive. The inversion of sensitivity trends did not persist into adulthood; *Elimia cahawbensis* was the most sensitive adult species/group, whereas the adult *Elimia* spp. was the least sensitive. Thus, in both juveniles and adults, the most sensitive species/groups to Ni and K were the least sensitive to Cl. Additionally, the exposure time altered the relative sensitivity across species differently across age classes. Juvenile trends were generally unaffected by increasing exposure time, except for *Elimia* spp. and *E. cahawbensis* exposed to K (Figure 1b). However, the 48-h LC50 for *Elimia* spp. is higher than that of *S. georgianus*, whereas the 96-h LC50 for *Elimia* spp. is lower than that of *S. georgianus* (Figure 2b). The same trend was seen between *E. cahawbensis* and *S.*

georgianus where *E. cahawbensis* had higher LC50 values at lower exposure times than *S. georgianus* (Figure 2c).

Our Cl results are relatively unique and may be a consequence of our experimental design. Gibson et al. (2018) reported Cl 96-h EC50 values of 190.60 and 3.41 mg/L Cl for two species of Alabama gastropods, adult *S. georgianus* and juvenile *Leptoxis ampla,* and 452.49 and 1538.45 mg/L Cl for two mussel species, *Hamiota perovalis* and *Cambarunio nebulosus*, respectively. The 96-hr LC50 of our adult *S. georgianus* was 3597.3 mg/L Cl, 18.9x higher than that proposed by Gibson et al. (2018). We believe this is because of the inflated Ca concentrations in our test water from our use of $CaCl_2$. Calcium, a main component of water hardness, is known to affect the toxicity of Cl (Elphick et al., 2011; Soucek et al., 2011), so the inflated LC50s in the present study may be an artifact of reduced bioavailability of Cl due to increased Ca concentrations. No water quality criteria hardness corrections exist that can adjust for the concentrations of Ca present in the current study, so we are unable to adjust our values for comparison with the literature. This is a major limitation of our study, and future research is necessary to better characterize the toxicity of Cl.

Current U.S. EPA water quality criteria have established CMC for Cl; however, these data are not representative of the taxa we examined. The CMC for Cl is 0.86 mg/L, and it was published in 1988 (U.S. EPA, 1988). Since then, numerous studies have been conducted that further characterize the toxicity of Cl across several taxa (Elphick et al., 2011; Soucek et al., 2011; Wang et al., 2017; Gibson et al., 2018). Furthermore, there is only one species of gastropod included in the Cl criteria: *Physa gyrina* (U.S. EPA, 1988). *Physa gyrina* is a pulmonate snail and is therefore not indicative of the freshwater gill-breathing snails that are more sensitive to Cl exposure (Gibson et al., 2018). Additionally, literature regarding Cl toxicity has proven the protective effects of hardness and the literature regarding *S. georgianus* suggests that current guidelines are not representative of gastropod toxicity (Gibson et al., 2018). Even so, the 96-h LC10 of *E. cahawbensis* is below current guidelines.

Environmental relevance and management implications

The toxicants in the present study are expected to enter the Cahaba River system via municipal and industrial effluents and agriculture runoff (State of Alabama Water Improvement Advisory Commission, 1949). Organisms are therefore exposed to these toxicants at different life stages and for varying times. We found that the sensitivity of these snail species to Ni, K, and Cl was influenced by organism age and exposure time. Whereas much toxicology research is conducted with surrogate organisms grown in laboratory conditions, we opted to use ecologically relevant field-collected snails. Additionally, the water quality (e.g., hardness) for the toxicity experiments mimicked the water quality of the Cahaba River, further reinforcing the ecologically relevant nature of the research.

The study generated a comprehensive toxicological dataset for three toxicants and three snail species endemic to the Cahaba River system. The dataset included lethal concentrations for different levels of effects (i.e., LC10, LC15, LC25, LC50) for exposure time ranging from 24-h to 96-h. The models describing the relationship between LC50 and exposure time can be used to predict toxicity of the toxicants based on exposure time. This is important because the concentrations of toxicants in the river system are expected to fluctuate over time depending on the intensity of runoff events.

More importantly, these data are directly applicable to developing modern water quality criteria for Alabama; the currently available criteria were established over three decades ago and did not accurately represent freshwater gastropod toxicity data, especially for Alabama endemic species. Furthermore, the current Ni criteria are not wholly protective of the endemic snails. Updating these criteria with environmentally relevant data may better reduce pollution-associated risk to these important species and protect better the rich biodiversity of the Cahaba River and other rivers in Alabama.

In conclusion, this study has determined the relative sensitivities of three endemic gastropods to pollutants that are expected to become increasingly more relevant with time, and we have shown how toxicity is affected by species and age. The study generated a comprehensive toxicity data set of the three pollutants to three Alabama endemic snail species for the first time. Although the data are useful for setting relevant water quality criteria for the aquatic ecosystem of Alabama, more research is needed, such as the influence of water quality on the toxicity of these pollutants, to help develop management strategies to better protect the uniquely biodiverse freshwater ecosystems of Alabama. The results also suggest that the current U.S. EPA guidelines are protective for Cl and approach potential risk with Ni. However, the study demonstrated acute toxicity of K to endemic gastropods with yet no federal or Alabama state K water quality criteria. This highlights the importance and the nescience of novel species gastropod toxicity.

Tables and Figures

Table 1. LC values for three toxicants (nickel, potassium, and chloride) over four exposure times (24, 48, 72, 96 h) to three juveniles species/groups (*Somatogyrus georgianus, Elimia cahawbensis, and Elimia* spp.).

Toxicant	Species	Time (h)	LC10 (LCL, UCL) mg/L	LC15 (LCL, UCL) mg/L	LC25 (LCL, UCL) mg/L	LC50 (LCL, UCL) mg/L
Nickel	*Somatogyrus georgianus*	24	NC	0.61 (0.51, 0.68)	0.69 (0.62, 0.78)	0.90 (0.82, 1.07)
		48	NC	0.57 (0.51, 0.59)	0.62 (0.57, 0.64)	0.74 (0.71, 0.76)
		72	NC	0.57 (0.50, 0.59)	0.62 (0.56, 0.63)	0.74 (0.70, 0.75)
		96	NC	0.35 (0.26, 0.82)	0.43 (0.28, 0.84)	0.61 (0.31, 0.89)
	Elimia cahawbensis	24	NC	2.74 (2.24, 3.54)	3.33 (2.48, 4.70)	> 4.41
		48	NC	2.32 (2.21, 2.40)	2.60 (2.45, 2.76)	3.30 (3.00, 3.66)
		72	NC	2.16 (1.90, 2.34)	2.43 (2.20, 2.58)	3.09 (2.94, 3.19)
		96	0.83 (0.52, 1.05)	1.08 (0.77, 1.31)	1.27 (0.98, 1.50)	1.72 (1.44, 2.04)
	Elimia spp.	24	NC	2.21 (1.87, -)	2.64 (2.07, -)	>2.86

		48	0.98 (0.72, 1.16)	1.07 (0.82, 1.25)	1.22 (0.99, 1.40)	1.55 (1.35, 1.78)
		72	0.82 (0.63, 0.97)	0.90 (0.70, 1.04)	1.01 (0.83, 1.16)	1.27 (1.10, 1.45)
		96	0.68 (0.52, 0.80)	0.74 (0.59, 0.86)	0.84 (0.69, 0.96)	1.05 (0.92, 1.21)
Potassium	*Somatogyrus georgianus*	24	17.75 (20.67, 14.18)	19.43 (22.44, 15.94)	22.20 (18.80, 25.52)	28.46 (24.75, 33.54)
		48	16.86 (13.54, 19.50)	18.40 (15.19, 21.11)	20.92 (17.85, 23.93)	26.57 (23.23, 31.38)
		72	16.86 (13.54, 19.50)	18.40 (15.19, 21.11)	20.92 (17.85, 23.93)	26.57 (23.23, 31.38)
		96	16.86 (13.54, 19.50)	18.40 (15.19, 21.11)	20.92 (17.85, 23.93)	26.57 (23.23, 31.38)
	Elimia cahawbensis	24	NC	NC	NC	NC
		48	692.80 (374.70, 1412.00)	959.00 (591.90, 3095.00)	1550.00 (913.40, 12,550.00)	3792.00 (1690.00, 206,900)
		72	72.85 (59.21, 84.47)	81.12 (67.80, 93.52)	95.06 (81.72, 110.10)	127.80 (110.30, 156.70)
		96	72.05 (65.09, 74.02)	73.17 (67.57, 76.14)	77.42 (72.54, 80.39)	88.04 (84.95, 91.02)
	Elimia spp.	24	NC	NC	NC	NC

		48	NC	NC	NC	NC
		72	74.05 (72.47, 74.95)	78.54 (76.18, 79.89)	87.54 (83.60, 89.79)	110.00 (102.20, 114.50)
		96	44.55 (15.85, 87.61)	59.93 (16.87, 78.36)	71.65 (39.86, 80.88)	91.43 (75.61, 97.59)
Chloride	*Somatogyrus georgianus*	24	2485.00 (843.50, 2835.00)	2624.00 (1414.00, 2954.00)	2901.00 (2173.00, 3192.00)	3595.00 (3109.00, 3489.00)
		48	1565.42 (821.15, 3677.12)	1978.90 (986.54, 3503.60)	2554.25 (1594.10, 3433.35)	3363.58 (2877.98, 3949.64)
		72	1167.25 (212.14, 1635.03)	1396.96 (276.21, 1811.65)	1856.39 (1172.99, 2361.75)	2937.31 (2699.07, 3346.54)
		96	1139.41 (242.77, 1637.96)	1327.35 (352.79, 1888.22)	1703.24 (979.65, 2426.84)	2697.07 (1514.53, 3611.14)
	Elimia cahawbensis	24	NC	NC	NC	NC
		48	NC	NC	NC	NC
		72	961.32 (613.89, 1157.03)	1026.81 (698.58, 1223.54)	1131.78 (837.95, 1340.70)	1356.84 (1118.16, 1672.17)
		96	507.50 (369.55, 604.53)	546.49 (412.35, 643.07)	609.60 (482.77, 707.45)	747.31 (633.28, 864.27)
	Elimia spp.	24	2330.12 (2290.78, 2428.46)	2470.61 (2411.60, 2618.12)	2751.58 (2653.24, 2997.44)	3454.03 (3257.34, 3945.74)

48	765.28 (550.49, 942.84)	868.98 (651.83, 1051.92)	1048.33 (830.39, 1245.70)	1487.14 (1251.96, 1777.77)
72	770.55 (587.21, 896.42)	831.38 (657.04, 956.17)	930.09 (770.80, 1058.32)	1146.36 (1003.03, 1323.24)
96	500.00 (409.17, 568.79)	529.92 (442.48, 598.94)	577.40 (494.88, 648.80)	677.54 (599.52, 765.74)

LCx = median lethal concentration that causes mortality in x% of a population

LCL = Lower Confidence Limit

UCL = Upper Confidence Limit

NC = Not Calculatedble

Table 2. Linear model coefficients describing the change in LC50 over time for three toxicants and three juvenile species/groups (*Somatogyrus georgianus, Elimia cahawbensis*, and *Elimia* spp.

Toxicant	Species/Group	Slope	Intercept	R-squared	p value
Nickel	*Somatogyrus georgianus*	-0.004	0.966	0.903	0.003
	Elimia cahawbensis	-0.033	5.073	0.847	0.129
	Elimia spp.	-0.01	2.044	0.994	0.018
Potassium	*Somatogyrus georgianus*	-0.024	28.461	0.600	0.001
	Elimia cahawbensis	-1.66	247.079	1.000	NA*
	Elimia spp.	-0.77	165.698	1.000	NA*
Chloride	*Somatogyrus georgianus*	-13	3928.255	0.985	<0.001
	Elimia cahawbensis	-25.4	3185.400	1.000	NA*
	Elimia spp.	-36.13	3858.830	0.840	0.034

Slope represents the change in sensitivity (mg/L of toxicant) for every one hour of exposure.

* p-value could not be calculated due to perfect fit (R^2 = 1.000)

LC50 = median lethal concentration that causes mortality in 50% of a population.

NA = Not applicable

Table 3. LC values for three toxicants (nickel, potassium, and chloride) over four exposure times (24, 48, 72, 96 h) to three adult species/groups (*Somatogyrus georgianus, Elimia cahawbensis, and Elimia* spp.).

Toxicant	Species	Time (h)	LC10 (LCL, UCL) mg/L	LC15 (LCL, UCL) mg/L	LC25 (LCL, UCL) mg/L	LC50 (LCL, UCL) mg/L
Nickel	*Somatogyrus georgianus*	24	1.05 (0.91, 1.12)	1.1 (0.97, 1.17)	1.2 (1.09, 1.26)	1.44 (1.38, 1.5)
		48	0.99 (0.72, 1.15)	1.03 (0.8, 1.18)	1.12 (0.95, 1.25)	1.33 (1.22, 1.42)
		72	0.77 (0.68, 0.85)	0.8 (0.73, 0.99)	0.86 (0.77, 1.23)	1.03 (0.83, 1.54)
		96	0.46 (0.29, 1.02)	0.56 (0.3, 0.94)	0.74 (0.35, 0.83)	0.84 (0.74, 1)
	Elimia cahawbensis	24	7.64 (4.18, 23.41)	>15.15	>15.15	>15.15
		48	3.79 (3.22, 4.45)	4.21 (3.63, 4.86)	5.03 (4.45, 5.61)	7.09 (6.52, 8.25)

		72	1.85 (1.53, 2.29)	2.07 (1.75, 2.49)	2.53 (2.21, 2.88)	3.86 (3.14, 4.87)
		96	1.15 (0.99, 2.29)	1.27 (1.02, 2.48)	1.5 (1.09, 2.87)	2.52 (0.78, 3.43)
	Elimia spp.	24	1.17 (0.82, 2.31)	1.44 (0.97, 3.07)	2.6 (0.4, NC)	>4.07
		48	0.58 (0.42, 0.88)	0.65 (0.45, 1)	0.82 (0.4, 1.2)	1.32 (0.76, 1.63)
		72	0.12 (0.03, 0.98)	0.2 (NC, 1.22)	0.35 (0.04, 1.34)	0.97 (NC, 1.28)
		96	0.12 (0.01, 0.94)	0.49 (NC, 0.59)	0.54 (0.22, 0.65)	0.69 (0.56, 1.1)
Potassium	*Somatogyrus georgianus**	24	48.68 (48.68, 48.68)	50.85 (50.85, 50.85)	55.17 (55.17, 55.17)	65.98 (65.98, 65.98)
		48	48.68 (48.68, 48.68)	50.85 (50.85, 50.85)	55.17 (55.17, 55.17)	65.98 (65.98, 65.98)

	72	48.68 (48.68, 48.68)	50.85 (50.85, 50.85)	55.17 (55.17, 55.17)	65.98 (65.98, 65.98)
	96	48.68 (48.68, 48.68)	50.85 (50.85, 50.85)	55.17 (55.17, 55.17)	65.98 (65.98, 65.98)
Elimia cahawbensis	24	>1900	>1900	>1900	>1900
	48	>1900	>1900	>1900	>1900
	72	76.43 (59.9, 126.04)	88.24 (63.44, 168.11)	143.76 (35.44, 266.13)	492.14 (492.14, 1841.09)
	96	37.03 (15.23, 56.36)	55.81 (29.45, 78.17)	71.23 (42.92, 96.37)	112.22 (80.69, 152.96)
Elimia spp.	24	480.81 (158.3, 1879.82)	1101.52 (447.1, 14206.9)	3746.34 (1187.94, 490898)	36667.5 (5259.48, 504325000)
	48	34.58 (19.17, 51.55)	46.34 (27.67, 66.28)	71.41 (47.16, 96.95)	159.8 (120.26, 208.58)

		72	11.3 (7.1, 14.93)	13.01 (8.62, 16.82)	16.02 (11.42, 20.2)	23.59 (18.51, 29.59)
		96	8.86 (5.15, 11.97)	10.27 (6.39, 13.53)	12.77 (8.71, 16.32)	19.16 (14.78, 24.28)
Chloride	*Somatogyrus georgianus*	24	5165.92 (4461.44, 5482.94)	5417.52 (4752.18, 5716.92)	5920.72 (5333.65, 6184.9)	7178.72 (6787.34, 7354.84)
		48	5004.18 (1635.41, 5660.85)	5264.76 (2850.74, 5884.95)	5785.93 (4417.86, 6333.16)	7088.87 (6176.81, 7453.68)
		72	2738.19 (2242.44, 3102.49)	2878.58 (2401.49, 3241.49)	3099.16 (2650.11, 3467.45)	3556.3 (3145.24, 3980.03)
		96	2610.78 (2265.5, 2766.16)	2734.1 (2408, 2880.84)	2980.73 (2693, 3110.21)	3597.3 (3405.48, 3683.62)
	Elimia cahawbensis	24	NC	NC	NC	NC
		48	2729.11 (1520.02, 3873.12)	3693.51 (2372.04, 5246.25)	5774.21 (4082.87, 9204.38)	13277.5 (5816.54, 34607.4)

	72	882.59 (581.44, 1125.97)	1008.8 (700.41, 1257.37)	1228.9 (916.86, 1488.23)	1775.19 (1462.14, 2110.32)
	96	835.76 (470.63, 1044.87)	910.19 (552.2, 1113)	1032.38 (696.54, 1226.47)	1305.53 (1043.91, 1511.63)
Elimia spp.	24	3099.15 (2950.26, 3288.65)	3430.02 (3206.69, 3714.27)	4091.76 (3719.54, 4565.51)	>9949
	48	2948.01 (2599.53, 3162.47)	3254.38 (2986.31, 3468.83)	3867.1 (3644.07, 4081.55)	>9949
	72	3718.53	3840.47	4027.88	4401.87
	96	2576.14 (NC, 2863.83)	2721.61 (2124.79, 2993.32)	3012.56 (2485.95, 3252.31)	3739.93 (3388.86, 3899.76)

* *Somatogyrus georgianus* exposed to potassium experienced a single mass mortality that was evenly distributed between replicates between the highest test concentrations resulting in identical LC values

LCx = median lethal concentration that causes mortality in x% of a population

LCL = Lower Confidence Limit

UCL = Upper Confidence Limit

NC = Not Calculated

Table 4. Linear model coefficients describing the change in LC50 over time for three toxicants and three adult species/groups (*Somatogyrus georgianus, Elimia cahawbensis*, and *Elimia* spp.

Toxicant	Species/Group	Slope	Intercept	R-squared	P-value
Nickel	*Somatogyrus georgianus*	-0.009	1.688	0.975	0.124
	Elimia cahawbensis	-0.095	11.347	0.946	0.149
	Elimia spp.	-0.013	1.931	0.996	0.04
Potassium	*Somatogyrus georgianus*†	0.00	65.980	NA	NA
	Elimia cahawbensis	-15.83	1631.900	1.000	NA*
	Elimia spp.	-2.93	278.477	0.603	0.233
Chloride	*Somatogyrus georgianus*	-59.49	8924.500	0.805	0.103
	Elimia cahawbensis	-249.40	23410.700	0.779	0.311
	Elimia spp.	-27.58	6387.690	1.000	NA*

Slope represents the change in sensitivity (mg/L of toxicant) for every one hour of exposure.

* p-value could not be calculated due to perfect fit (R^2 = 1.000)

† *Somatogyrus georgianus* exposed to potassium experienced a single mass mortality event that resulted in complete mortality of the two highest test concentrations after 24 hours.

LC50 = median lethal concentration that causes mortality in 50% of a population.

NA = Not applicable

Table 5. Lethal accumulation (LA) concentrations of nickel (mg/g ww Ni) in three juvenile snails species/groups (*Somatogyrus georgianus, Elimia cahawbensis,* and *Elimia* spp.) after 96-hour exposure (95% confidence limits in parentheses).

Species	LA10	LA15	LA25	LA50
Somatogyrus georgianus	1.8 (1.2, 2.9)	2.7 (1.8, 8.0)	7.0 (2.3, 7.8)	9.3 (8.7, 9.7)
Elimia cahawbensis	72.5 (26.8, 104.0)	84.9 (37.1, 117.5)	106.9 (58.8, 143.6)	164.6 (119.2, 242.7)
Elimia spp.	22.8 (21.1, 29.8)	25.3 (22.7, 28.5)	27.4 (26.4, 29.2)	31.2 (30.4, 32.4)

LAx = concentration in tissue resulting in mortality in x% of the population

ww = wet weight

Table 6. Pearson correlation coefficients between lethal concentrations (LC) and lethal accumulation (LA) values for nickel (Ni) across three juvenile snail species/groups.

C	*LA50*	*LC50*	*LA25*	*LC25*	*LA15*	*LC15*
LA50	1					
LC50	**0.96**	1				
LA25	1.00	0.98	1			
LC25	0.93	1.00	**0.95**	1		
LA15	0.99	0.99	1.00	0.97	1	
LC15	0.91	0.99	0.93	1.00	**0.96**	1

Bolded values are of interest to the present study.

LCx = median lethal concentration that causes mortality in x% of a population

Lax = concentration in tissue resulting in mortality in x% of the population

Table 7. Pearson correlation coefficients between potassium concentrations in test water (K_W) and tissue cation concentrations (K_t, Ca_t, Mg_t, and Na_t) in three juvenile snail species/groups (*Somatogyrus georgianus, Elimia cahawbensis,* and *Elimia* spp.) across increasing potassium test concentrations.

		K_W	K_t	Ca_t	Mg_t	Na_t
Somatogyrus georgianus	K_W	1				
	K_t	-0.55	1			
	Ca_t	-0.19	0.49	1		
	Mg_t	-0.67	0.85	0.67	1	
	Na_t	-0.38	0.80	0.70	0.81	1
Elimia cahawbensis	K_W	1				
	K_t	0.11	1			
	Ca_t	-0.67	0.56	1		
	Mg_t	-0.58	0.64	0.98	1	
	Na_t	-0.54	0.60	0.94	0.98	1
Elimia spp	K_W	1				
	K_t	0.91	1			
	Ca_t	-0.14	0.16	1		
	Mg_t	-0.66	-0.37	0.79	1	
	Na_t	-0.50	-0.39	0.63	0.73	1

K_W = potassium concentration in test water. K_t = potassium concentration in tissue. Ca_t = calcium concentration in tissue. Mg_t = magnesium concentration in tissue. Na_t = sodium concentration in tissue.

Table 8. Lethal accumulation (LA) concentrations of nickel (mg/kg dw Ni) in three adult snails species/groups (*Somatogyrus georgianus, Elimia cahawbensis,* and *Elimia* spp.) after 96 hour exposure (95% confidence limits in parentheses).

Toxicant	Species	LA10 mg/kg dw	LA15 mg/kg dw	LA25 mg/kg dw	LA50 mg/kg dw
Nickel	*Somatogyrus georgianus*	62.45 (52.26, 95.04)	68.27 (52.99, 94.46)	79.08 (55.35, 94.20)	95.75 (77.08, 106.03)
	Elimia cahawbensis	218.80 (152.69, 758.70)	273.89 (174.73, 858.93)	384.07 (218.80, 1059.4)	893.65 (209.37, 1378.73)
	Elimia spp.*	NC	NC	NC	NC

* Elimia spp. LA values could not be analyzed due to high Ca concentrations interfering with ICP-MS analysis

LAx = concentration in tissue resulting in mortality in x% of the population

dw = dry weight

NC = Not calculated

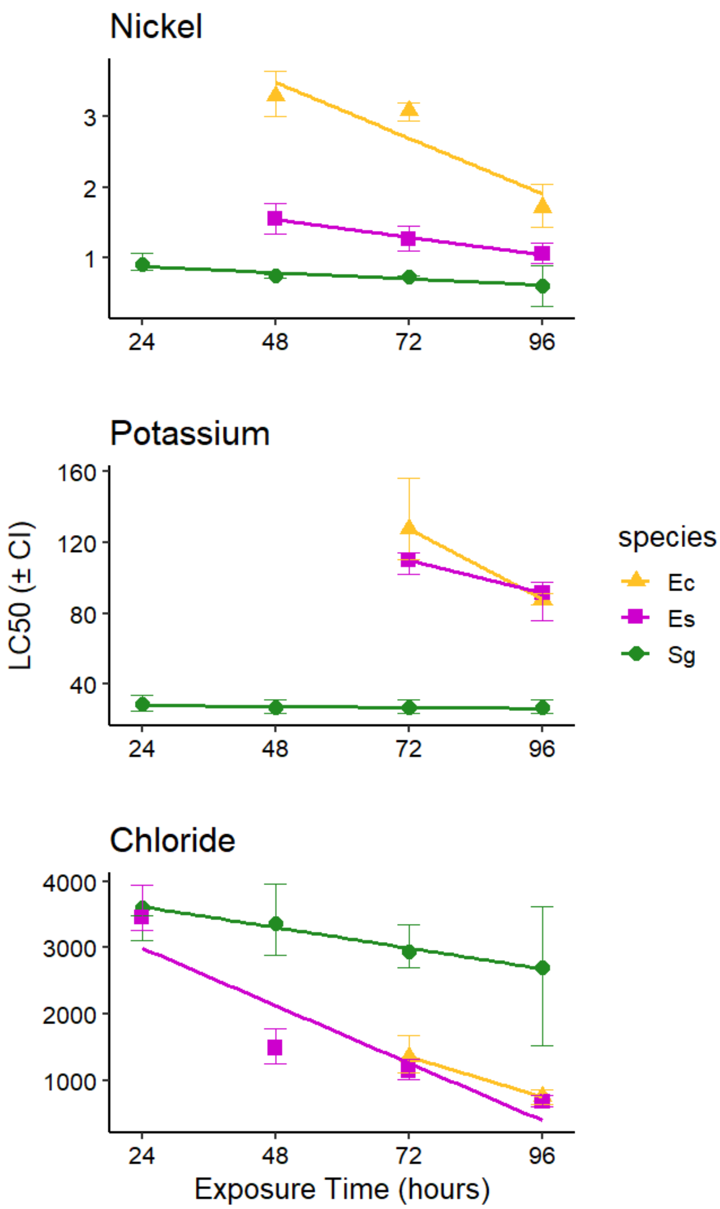

Figure 1. LC50 values for three juvenile snail species/groups (*Somatogyrus georgianus, Elimia cahawbensis,* and *Elimia* spp.) exposed to nickel (Ni), potassium (K), and chloride (Cl) across four exposure time points (24, 48, 72, and 96 h). Lines represent the best fit on each linear model, and the error bars represent the 95% confidence intervals. LC50 is the median lethal concentration that causes mortality in

50% of a population. CI is the confidence interval of the data. Ec, Es, and Sg are *Elimia cahawbensis, Elimia* spp., and *Somatogyrus georgianus* respectively.

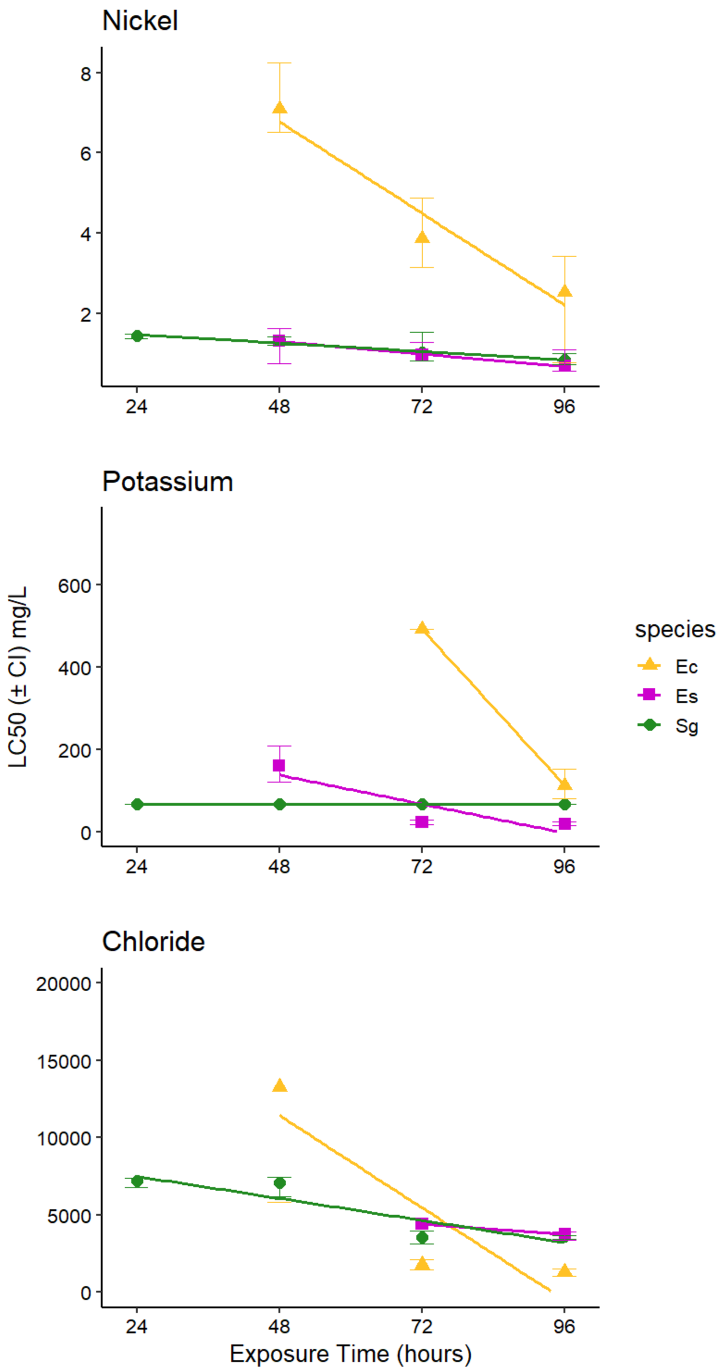

Figure 2. LC50 values for three adult snail species/groups (*Somatogyrus georgianus, Elimia cahawbensis,* and *Elimia* spp.) exposed to nickel (Ni), potassium (K), and chloride (Cl) across four exposure time points (24, 48, 72, and 96 h). Lines represent

the best fit of each linear model, and the error bars represent the 95% confidence intervals. LC50 is the median lethal concentration that causes mortality in 50% of a population. CI is the confidence interval of the data. Ec, Es, and Sg are *Elimia cahawbensis, Elimia* spp., and *Somatogyrus georgianus* respectively.

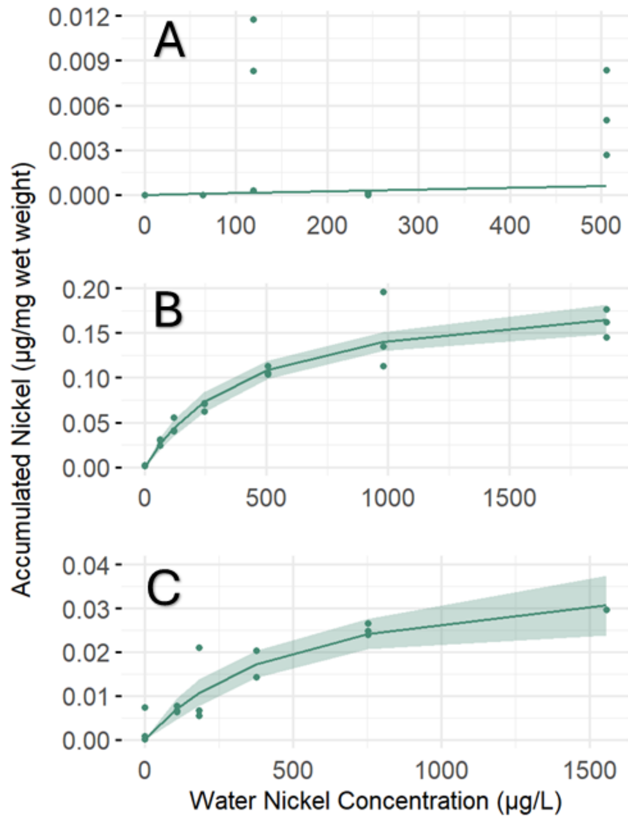

Figure 3. Michaelis-Menten models describing the relationship between ambient nickel concentration in test water (µg/L) and the accumulated nickel in the whole body (µg/mg wet weight) of three juvenile snail species/groups: (**A**) *Somatogyrus georgianus,* (**B**) *Elimia cahawbensis,* and (**C**) *Elimia* spp. Shaded area represents the 95% confidence. C_{MAX} = maximum accumulation concentration. K_M = ambient nickel concentration at which 50% tissue saturation is achieved. Confidence intervals could not be calculated for *S. georgianus*.

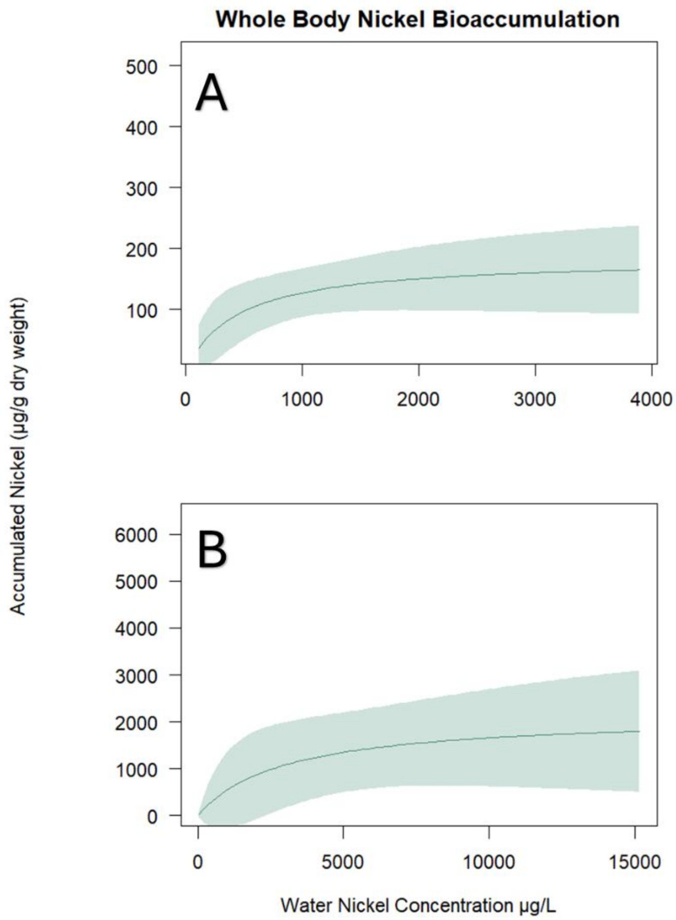

Figure 4. Michaelis-Menten models describing the relationship between ambient nickel concentration in test water (µg/L) and the accumulated nickel in the whole body (µg/g dry weight) of two adult snail species/groups: (**A**) *Somatogyrus georgianus and* (**B**) *Elimia cahawbensis*. Shaded area represents the 95% confidence. C_{MAX} = maximum accumulation concentration. K_M = ambient nickel concentration at which 50% tissue saturation is achieved.

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Appendix A

Table A1: Water quality parameters measured during juvenile nickel, potassium, and chloride toxicity experiments for three species of freshwater gastropod (*Somatogyrus georgianus*, *Elimia cahawbensis*, and *Elimia* spp.). Values are presented as mean (± standard deviation).

Toxicant	Species	Hardness mg/L Ca	Alkalinity mg/L CaCO3	Salinity g/L	Temperature °C	Dissolved Oxygen mg/L O2	pH
Nickel	*Somatogyrus georgianus*	96 (±0)	72 (±3.27)	0.06 (±0.05)	23.8 (±0.9)	5.54 (±0.96)	7.63 (±0.39)
	Elimia cahawbensis	96 (±0)	72 (±3.27)	0.05 (±0.05)	23.0 (±1.7)	6.93 (±0.64)	7.94 (±0.23)
	Elimia spp.	96 (±0)	75 (±5)	0.06 (±0.05)	24.2 (±0.7)	5.45 (±0.90)	7.68 (±0.36)
Potassium	*Somatogyrus georgianus*	88 (±9)	83 (±9)	0.18 (±0.15)	23.6 (±1.6)	5.71 (±0.33)	7.38 (±0.36)
	Elimia cahawbensis	73 (±5)	63 (±8)	0.53 (±0.49)	24.2 (±1.5)	5.77 (±0.50)	7.61 (±0.34)
	Elimia spp.	76 (±5)	63 (±8)	0.53 (±0.49)	24.2 (±1.5)	5.77 (±0.50)	7.61 (±0.34)
Chloride	*Somatogyrus georgianus*	NC*	67 (±7)	1.79 (±2.03)	24 (±0.2)	6.08 (±0.35)	6.79 (±0.51)
	Elimia cahawbensis	NC*	78 (±5)	1.77 (±2.02)	24.9 (±0.4)	5.45 (±0.69)	6.66 (±0.54)
	Elimia spp.	NC*	76 (±5)	1.76 (±2.02)	25.32(±0.2)	5.54 (±0.20)	6.65 (±0.54)

* Hardness could not be calculated through titration because of the addition of calcium chloride test toxicant.

NC = Not Calculated

Table A2: Water quality parameters measured during adult nickel, potassium, and chloride toxicity experiments for three species of freshwater gastropod (*Somatogyrus georgianus*, *Elimia cahawbensis*, and *Elimia* spp.). Values are presented as mean (± standard deviation).

Toxicant	Species	Hardness mg/L Ca*	Alkalinity mg/L CaCO3*	Salinity g/L	Temperature °C	Dissolved Oxygen mg/L O2	pH
Nickel	*Somatogyrus georgianus*	111 (±14)	NC	0.10 (±0.064)	25.9 (±0.4)	6.82 (±0.36)	7.5 (±0.1)
	Elimia cahawbensis	126 (±6)	24 (± 2)	0.18 (±0.031)	21.1 (±0.6)	6.78 (±0.57)	6.7 (±0.3)
	Elimia spp.	88 (±11)	NC	0.11 (±0.052)	25.5 (±0.3)	6.90 (±0.49)	7.6 (±0.1)
Potassium	*Somatogyrus georgianus*	126 (±27)	NC	0.49 (±0.43)	25.8 (±0.4)	6.60 (±0.55)	7.5 (±0.1)
	Elimia cahawbensis	121 (±8)	27 (± 8)	0.91 (±0.92)	21.3 (±0.3)	6.90 (±0.47)	7.0 (±0.2)
	Elimia spp.	60 (±17)**	NC	0.93 (±0.95)	25.7 (±0.3)	6.77 (±0.47)	7.7 (±0.03)
Chloride	*Somatogyrus georgianus*	NC†	NC	3.53 (±4.30)	25.8 (±0.5)	6.27 (±0.52)	7.6 (±0.2)
	Elimia cahawbensis	NC†	NC	3.85 (±3.83)	19.7 (±0.1)	6.5 (±0.33)	7.1 (±0.3)
	Elimia spp.	NC†	NC	3.20 (±4.10)	25.3 (±0.6)	5.47 (±0.24)	7.8 (±0.1)

* Alkalinity data collection was impeded during adult toxicity trials

† Hardness could not be calculated through titration because of the addition of calcium chloride test toxicant.

NC = Not Calculated

Table A3: Juvenile nickel exposure concentrations, bioaccumulation, and ion concentrations in ambient water and tissue of three freshwater gastropod species (*Somatogyrus georgianus*, *Elimia cahawbensis*, and *Elimia* spp.).

	Species	Treatment	Nominal (mg/L Ni)	Measured (mg/L Ni)	Accumulation (µg/g w.w. Ni)	Water Concentrations (mg/L)				Tissue Concentrations (mg/g ww) *			
						Ca	Mg	K	Na	Ca	Mg	K	Na
Nickel	*Somatogyrus georgianus*	Control	0.00	0.00	B/D	12.3	11.5	1.6	26.8	133	0.09	0.41	BD
		t1	0.06	0.06	B/D	13.6	12.6	6.5	29.8	119	BD	0.37	BD
		t2	0.13	0.12	6.78	13.7	12.7	2.0	29.2	114	4.49	2.51	8.45
		t3	0.25	0.24	0.05	13.4	12.4	1.8	28.7	90	0.97	0.51	1.72
		t4	0.50	0.51	3.91	13.7	12.6	1.9	28.6	124	BD	0.51	BD
		t5	1.00	0.98	26.84	13.5	12.6	1.9	28.8	156	BD	0.36	BD
		t6	2.00	1.90	13.66	13.5	10.2	1.9	28.9	152	BD	0.52	BD
	Elimia cahawbensis	Control	0.00	0.00	3.52	12.3	11.5	1.6	26.8	5.29	0.48	0.36	1.96
		t1	0.06	0.06	31.58	13.6	12.6	6.5	29.8	3.43	0.40	0.30	0.21
		t2	0.13	0.12	49.54	13.7	12.7	2.0	29.2	4.63	0.37	0.28	0.46
		t3	0.25	0.24	64.94	13.4	12.4	1.8	28.7	2.39	0.38	0.27	0.14
		t4	0.50	0.51	13.34	13.7	12.6	1.9	28.6	2.46	0.30	0.23	0.15
		t5	1.00	0.98	108.47	13.5	12.6	1.9	28.8	3.60	0.27	0.27	1.43
		t6	2.00	1.90	195.39	13.5	10.2	1.9	28.9	1.94	0.17	0.16	0.06
		t7	4.00	4.41	265.33	14.9	11.4	3.1	32.0	2.42	0.14	0.15	1.16
	Elimia spp.	Control	0.00	0.00	3.00	14.4	12.5	1.8	28.2	0.58	0.11	0.07	0.05
		t1	0.09	0.11	7.66	14.9	13.0	1.9	29.2	0.53	0.10	0.06	0.04
		t2	0.19	0.18	12.18	13.5	12.3	1.7	28.0	0.38	0.12	0.06	0.06
		t3	0.38	0.38	17.81	13.8	12.4	1.7	28.0	0.41	0.15	0.07	0.06
		t4	0.75	0.75	26.12	14.0	12.5	1.7	28.6	1.34	0.12	0.05	0.04

	t5	1.50	1.56	60.83	13.8	12.5	1.7	28.2	3.62	0.10	0.11	0.04
	t6	3.00	2.86	37.97	13.5	10.0	1.8	27.8	9.11	0.30	0.19	0.16

BD = Concentration was below the detection limit of our inductively coupled plasma mass spectrometer

Table A4: Adult nickel exposure concentrations, bioaccumulation, and ion concentrations in ambient water and tissue of three freshwater gastropod species (*Somatogyrus georgianus*, *Elimia cahawbensis*, and *Elimia* spp.).

Species	Treatment	Nominal (mg/L Ni)	Measured (g/L Ni)	Accumulation (mg/kg dw Ni)	Water Concentrations (mg/L)				Tissue Concentrations (mg/kg dw)			
					Ca	Mg	K	Na	Ca	Mg	K	Na
Somatogyrus georgianus	Control	0.00	BD	BD	9.1	14.6	3.2	34.1	325	0.6	0.32	2.00
	t1	0.13	110.00	34.64	14.5	20.4	3.7	47.2	297	0.5	0.26	1.77
	t2	0.25	262.28	50.81	14.0	19.6	3.4	45.1	350	1.3	0.53	2.76
	t3	0.50	728.26	77.97	14.7	21.1	3.9	49.0	384	1.1	0.46	2.76
	t4	0.75	986.56	188.28	14.2	20.2	3.7	46.1	460	2.0	0.89	3.42
	t5	1.50	1759.62	125.39	12.7	18.9	3.2	42.1	335	0.5	0.22	1.81
	t6	3.00	3890.52	148.23	12.6	19.3	3.2	43.4	311	0.3	0.10	1.58
Elimia cahawbensis	Control	0.00	BD	BD	13.5	20.3	5.8	10.7	20	17.3	8.5	5.56
	t1	0.25	40.90	31.82	14.8	20.3	3.4	11.7	20	13.8	6.9	4.42
	t2	0.50	482.29	130.34	14.6	21.2	4.2	18.1	26	16.4	7.4	4.92
	t3	1.00	917.49	149.69	14.7	21.8	4.0	18.2	29	13.6	6.8	3.75
	t4	2.00	1693.73	710.05	14.5	21.2	4.6	16.8	492	77.9	101.4	48.8
	t5	4.00	3518.31	1530.20	12.9	20.1	3.4	14.2	127	45.4	55.8	5.09
	t6	8.00	7642.62	1620.74	11.4	21.0	3.1	11.4	159	76.0	59.74	9.94
	t7	16.00	15151.38	1653.65	6.9	20.1	1.9	5.9	235	56.2	45.22	8.88
Elimia spp.*	Control	0.00	BD	NC	9.1	16.5	2.8	38.5	NC	NC	NC	NC
	t1	0.13	30.19	NC	8.9	16.8	3.0	36.9	NC	NC	NC	NC
	t2	0.25	118.57	NC	8.1	16.6	2.9	35.1	NC	NC	NC	NC
	t3	0.50	479.14	NC	8.0	16.7	2.9	35.7	NC	NC	NC	NC
	t4	1.00	722.19	NC	6.8	19.4	2.7	35.2	NC	NC	NC	NC
	t5	2.00	1713.66	NC	4.7	15.9	2.12	28.3	NC	NC	NC	NC
	t6	4.00	4064.68	NC	2.4	18.6	1.79	29.2	NC	NC	NC	NC

* *Elimia* spp. nickel accumulation could not be measured because of high concentrations of calcium in samples

BD = Concentration was below the detection limit of our inductively coupled plasma mass spectrometer

NC = Not Calculated

Table A5: Juvenile potassium exposure concentrations, bioaccumulation, and ion concentrations in ambient water and tissue of three freshwater gastropod species (*Somatogyrus georgianus*, *Elimia cahawbensis*, and *Elimia* spp.).

Species	Treatment	Nominal Concentration (mg/L)	Water Concentration (mg/L)				Tissue Concentrations (mg/g ww)			
			K	Ca	Mg	Na	K	Ca	Mg	Na
Somatogyrus georgianus	Control	0.00	2.41	12.15	12.94	29.27	0.37	199.50	0.14	0.14
	t1	1.00	3.58	13.30	14.18	32.34	0.35	175.29	0.11	-0.04
	t2	10.00	21.18	12.88	13.83	31.85	0.33	135.61	0.03	-0.04
	t3	25.00	21.36	12.58	10.66	31.49	0.42	180.31	0.07	-0.04
	t4	50.00	51.14	12.00	10.34	27.68	0.03	145.48	-0.03	-0.25
	t5	100.00	126.49	12.43	10.82	24.83	0.10	166.90	-0.05	-0.10
	t6	200.00	220.44	11.91	10.36	24.09	0.19	163.33	-0.01	-0.10
Elimia cahawbensis	Control	0.00	1.84	11.80	13.27	30.07	0.15	1.48	0.34	0.08
	t1	10.00	68.21	12.81	13.19	30.77	0.25	1.42	0.31	0.05
	t2	50.00	61.13	13.16	11.40	32.06	0.18	1.43	0.24	0.02
	t3	100.00	106.46	12.14	10.40	24.51	0.08	0.45	0.01	-0.06
	t4	250.00	315.60	12.88	10.88	25.07	0.09	0.51	0.01	-0.08
	t5	500.00	589.11	11.84	9.87	23.50	0.14	0.48	0.04	-0.03
	t6	1000.00	1150.28	9.64	8.43	20.47	0.20	0.40	0.04	-0.05
Elimia spp.	Control	0.00	2.38	12.15	12.94	29.27	0.09	0.95	0.24	0.28
	t1	10.00	13.80	12.51	13.30	30.66	0.11	0.89	0.23	0.07
	t2	50.00	65.05	13.99	11.61	32.51	0.15	0.81	0.22	0.05
	t3	100.00	131.00	12.98	11.08	25.67	0.10	0.37	0.12	0.03
	t4	250.00	321.24	13.38	11.52	26.17	0.09	0.43	0.07	-0.01
	t5	500.00	567.66	11.39	9.81	22.98	0.22	0.73	0.11	0.01
	t6	1000.00	1134.38	9.90	8.52	20.91	0.30	0.73	0.10	0.01

Table A6: Adult potassium exposure concentrations, bioaccumulation, and ion concentrations in ambient water and tissue of three freshwater gastropod species (*Somatogyrus georgianus*, *Elimia cahawbensis*, and *Elimia* spp.).

Species	Treatment	Nominal Concentration (mg/L)	Water Concentration (mg/L)			
			K	Ca	Mg	Na
Somatogyrus georgianus	Control	0	3.18	9.11	14.55	34.09
	t1	5	14.00	18.19	25.75	60.10
	t2	25	44.36	15.49	23.31	53.25
	t3	50	87.59	17.50	26.41	59.74
	t4	100	162.85	15.23	23.39	53.43
	t5	200	279.73	12.54	23.24	45.67
	t6	400	494.32	5.95	21.28	35.20
Elimia cahawbensis	Control	0	4.89	12.18	16.19	10.02
	t1	50	52.81	15.19	19.61	9.85
	t2	100	100.05	16.23	19.32	9.53
	t3	250	274.87	18.79	20.79	9.20
	t4	500	492.14	14.21	17.85	6.36
	t5	1000	1019.63	12.60	18.28	2.62
	t6	2000	1900.30	5.96	15.61	BD
Elimia spp.	Control	0	2.58	10.21	14.49	31.34
	t1	13	12.29	9.15	13.32	28.48
	t2	50	42.40	6.56	10.66	22.51
	t3	100	104.51	6.67	11.27	22.01
	t4	250	238.49	5.52	11.29	21.52
	t5	500	444.00	3.62	9.87	17.82
	t6	1000	776.84	0.40	9.48	14.76
	t7	2000	1891.49	BD	7.64	6.90

Table A7: Juvenile chloride exposure concentrations and ion concentrations in ambient water during toxicity trials for three freshwater gastropod species (*Somatogyrus georgianus*, *Elimia cahawbensis*, and *Elimia* spp.).

Species	Treatment	Nominal Concentration (mg/L)	Measured Cl Concentration (mg/L)	Water Concentrations (mg/L)			
				Ca	Mg	K	Na
Somatogyrus georgianus	Control	0.00	4.93	13.42	10.78	2.26	26.54
	t1	100.00	100.14	62.69	9.90	3.90	27.41
	t2	250.00	254.54	139.84	9.87	2.01	24.04
	t3	500.00	511.32	270.45	9.97	2.01	24.37
	t4	1000.00	1014.11	515.55	9.80	1.66	23.53
	t5	2000.00	2392.38	1017.55	9.51	1.28	22.69
	t6	4000.00	4982.24	1972.66	8.87	0.35	21.00
Elimia cahawbensis	Control	0.00	4.93	11.14	8.84	2.10	21.70
	t1	100.00	98.67	57.32	8.13	1.82	20.04
	t2	250.00	248.54	125.24	8.26	1.76	20.26
	t3	500.00	510.37	244.95	8.30	1.68	20.40
	t4	1000.00	1095.82	479.92	8.17	1.47	20.16
	t5	2000.00	2217.18	914.35	7.91	0.73	18.82
	t6	4000.00	4971.04	1892.93	7.74	-0.41	18.09
Elimia spp.	Control	0.00	4.93	11.47	8.18	2.08	20.30
	t1	100.00	97.25	55.23	7.28	1.75	17.82
	t2	250.00	237.12	119.16	7.23	1.71	17.87
	t3	500.00	474.61	224.54	7.13	1.83	17.62
	t4	1000.00	967.23	458.26	7.24	1.22	17.82
	t5	2000.00	2049.14	921.95	7.11	0.41	16.99
	t6	4000.00	4671.60	1729.20	6.81	-0.88	15.58

Table A8: Adult chloride exposure concentrations for three freshwater gastropod species (*Somatogyrus georgianus*, *Elimia cahawbensis*, and *Elimia* spp.).

Species	Treatment	Nominal Concentration (mg/L)	Measured Concentration (mg/L)
Somatogyrus georgianus	Control	0	3.38
	t1	250	302.39
	t2	500	604.60
	t3	1000	1277.41
	t4	2000	2446.37
	t5	4000	4830.45
	t6	8000	9694.71
Elimia cahawbensis	Control	0	3.38
	t1	250	302.39
	t2	500	604.60
	t3	1000	1277.41
	t4	2000	2446.37
	t5	4000	4830.45
	t6	8000	9694.71
Elimia spp.	Control	0	2.44
	t1	250	286.90
	t2	500	594.35
	t3	1000	1411.65
	t4	2000	2437.41
	t5	4000	5194.67
	t6	8000	9949.06

Appendix B

Table B1: Water Quality of the Cahaba River used for determining our Experimental Parameters

Site	Hardness mg/L Ca	Salinity g/L	Temperature °C	Dissolved Oxygen mg/L O2	pH
Cahaba River	96	0.1	26.7	6.8	7
Sixmile Creek	84	0.1	24.1	7.6	8

Table B2: Snail weights and operculum lengths from three species of freshwater gastropod endemic to the Cahaba River Basin

Species	Organism ID	Operculum (mm)†	Weight (mg)
*Somatogyrus georgianus**	Sg 01	0.61	0.631
	Sg 02	0.61	0.631
	Sg 03	0.71	0.631
	Sg 04	0.62	0.631
	Sg 05	0.59	0.631
	Sg 06	0.64	0.631
	Sg 07	0.87	0.631
	Sg 08	0.91	0.631
	Sg 09	0.7	0.631
	Sg 10	0.8	0.631
	Sg 11	0.87	0.957
	Sg 12	0.67	0.957
	Sg 13	0.92	0.957
	Sg 14	0.8	0.957
	Sg 15	0.56	0.957
	Sg 16	0.82	0.957
	Sg 17	0.96	0.957
	Sg 18	0.62	0.957
	Sg 19	0.67	0.957
	Sg 20	0.69	0.957
	Sg 21	0.86	0.568
	Sg 22	0.61	0.568
	Sg 23	0.87	0.568
	Sg 24	0.72	0.568
	Sg 25	0.76	0.568
	Sg 26	0.77	0.568

	Sg 27	0.71	0.568
	Sg 28	0.86	0.568
	Sg 29	0.76	0.568
	Sg 30	0.7	0.568
Elimia cahawbensis	Ec 01	4.9	151.61
	Ec 02	2.07	18.6
	Ec 03	2.95	52.88
	Ec 04	3.68	76.49
	Ec 05	2.53	31.5
	Ec 06	3.71	78.88
	Ec 07	3.81	93.75
	Ec 08	3.89	89.86
	Ec 09	4.83	113.28
	Ec 10	4.64	111.38
	Ec 11	3.08	44.63
	Ec 12	3.55	80.37
	Ec 13	4.32	129.93
	Ec 14	3.95	74.9
	Ec 15	4.73	112.04
	Ec 16	4.93	128.35
	Ec 17	4.14	62.08
	Ec 18	3.25	73.61
	Ec 19	3.65	63.78
	Ec 20	4.64	86.84
	Ec 21	2.29	17.83
	Ec 22	3.56	64.7
	Ec 23	5.85	166.9
	Ec 24	4.02	76.44
	Ec 25	4.79	118.95
	Ec 26	3.95	62.94

	Ec 27	4.1	94.14
	Ec 28	3.8	47.9
	Ec 29	3.48	54.11
	Ec 30	3.32	66.2
Elimia spp.	Es 01	4.32	122.66
	Es 02	6.9	99.33
	Es 03	4.66	87.01
	Es 04	4.67	88.88
	Es 05	4.13	111.77
	Es 06	3.46	95.95
	Es 07	5.68	197.45
	Es 08	5.59	179.4
	Es 09	3.91	57.77
	Es 10	4.23	73.33
	Es 11	4.33	117.6
	Es 12	3.39	66.07
	Es 13	5.05	107.67
	Es 14	4.62	142.07
	Es 15	4.78	113.62
	Es 16	3.96	78.79
	Es 17	3.88	68.04
	Es 18	3.31	69.36
	Es 19	4.4	65.8
	Es 20	4.18	76.74
	Es 21	4.02	93.51
	Es 22	4.86	120.56
	Es 23	4.06	100.36
	Es 24	4.88	120.95
	Es 25	3.21	59.45
	Es 26	4.27	113.3

Es 27	3.95	50.43
Es 28	4.31	80.6
Es 29	3.97	82.64
Es 30	5.41	128.02

**S. georgianus* weights were pooled averages among 3 groups of 10 snails: Sg 1 – Sg 10, Sg 11 – Sg 20, and Sg 21 – Sg 30. Individual snails were too light to accurately weigh.

†Operculum width refers to the anterior-posterior length of the operculum

Sg = *Somatogyrus georgianus*

Ec = *Elimia cahawbensis*

Es = *Elimia* spp.