Physella acuta growth in response to varying concentrations of Zinc

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Abstract:

Bladder snails*, Physella acuta,* are well-known for their fast maturation and reproduction as well as having higher tolerance to heavy-metal polluted stream. We investigated the effects of heavy metals, specifically zinc, on the growth of *P. acuta* by exposing them to 3 different zinc concentrations (200, 400, and 800 µg/L) and a negative control (0 µg/L) for 20 days. We quantified the shell length (SL) and body mass (BM) growth rate of *P. acuta* as a proxy of the effect of zinc concentrations. The 800 µg/L zinc treatment yielded both the highest mean growth rate of SL (0.0466±0.0013 cm/day) and BM (0.095±0.007 g/day). A general trend of increasing SL and BM growth rates of *P. acuta* was observed with increasing zinc concentrations. Statistical tests proved significance in variation in both BM (p=.001) and SL (p=.015) growth rates, while only 800µg/L treatment against the control showed significance on SL and BM growth rates (p=.001, p=.01). Furthermore, survival rates varied between the highest 76.7% in control and the lowest 28% in 800 µg/L treatment. The cause of mortality and therefore varying survival rates are not specified with possibilities including but not limited to; acclimation, overfeeding, and handling. Any egg masses observed were recorded by count and size (small, medium, large). Overall, the varying concentrations of zinc in this experiment did not negatively impact *P. acuta* as they were still able to thrive, reproduce with viable egg masses, and adapt to the varying levels of Zinc.

Introduction:

Zinc (Zn) is one metal that is found in a plethora within the Earth's crust and is reported to be an essential element for all living organisms. (Hussain et al., 2022). Zinc is typically found within soil and water bodies, having been introduced through the natural action of weathering or from release of industrial liquid waste. (Hussain et al., 2022). Fine sediment and low amounts of trace metals occur naturally in aquatic ecosystems; however, human activities increase these inputs resulting in low abundances of aquatic organisms at the affected sites (Dabney et al., 2018). Zinc is one of the metals that can be found in these streams in an array of amounts. Although intake of zinc is normal, excessive amounts can cause soil and microbial diversity to alter; affecting bioavailability and absorption of other metals present (Hussain et al., 2022).

The objective of this paper is to explore varying concentrations of zinc and its effect on shell length in centimeters and weight in grams of the bladder snails, *Physella acuta*. It is vital to understand what levels of zinc produce lethal effects as the entire ecosystem is impacted. Byzitter et al. (2012) reports that changes in the snail's activity and respiration are more sensitive to pollutants and, therefore, it would be an early indicator of harmful ecological impacts. *P. acuta* is known for its fast maturation and reproduction; they have been observed to grow 0.4mm per week in situ at 25℃ (Brackenbury & Appleton, 1991). *P. acuta* has a higher tolerance than other species to heavy metal pollution, thus, allowing it to be a model organism to study the lethal concentration of pollutants in aquatic life (Spyra et al., 2019).

In this study, we hypothesized that *P. acuta* experiencing heavy metal stress exhibits a negative impact on their growth. The negative impacts would vary depending on the zinc concentration as well as the amount of zinc uptaken by the snails. While zinc is an essential metal for living organisms, it has a dose-dependent sublethal action on snail growth at lower dosages (< 4000μg/g) (Gomot-De Vaufleury, 2000). El-Gendy et al. (2010) mentioned that the growth inhibitory effect of Zn was significant throughout their experiment, which supported the notion that a negative impact on growth would occur. Hickey (2019) conducted zinc toxicity tests on some *P.acuta,* which has adapted to heavily polluted regions, and found decreasing trends in growth, reproduction, and survival with elevated zinc concentration in water. We expected the snails receiving a higher dose of zinc treatment would have decreased shell length growth and body weight growth.

Method:

This study consisted of 3 treatments of different zinc concentrations (200, 400, and 800 μg/L Zn) and a control (0μg/L Zn). Each treatment and control was triplicated from October 27 to November 17, 2022. The chemical used in this experiment was zinc chloride. Water used for snail acclimation and zinc toxicity tests was the University of British Columbia (UBC) tap water, which originated from Sasamat Reservoir. The water was dechlorinated prior to use.

Figure 1. Bladder snails, *Physella acuta,* (pointed by yellow arrows) in the member's home aquarium.

Physella acuta, aged 1 to 7 weeks, were collected (N = 56) from a home aquarium of one group member (Figure 1). These snails spawned among the store-bought plants in the aquarium, and they had never been exposed to zinc or other heavy metals prior to the experiment. They were placed in a collection bucket filled with aquarium water and brought to the BIOL342 lab.

Snail acclimation

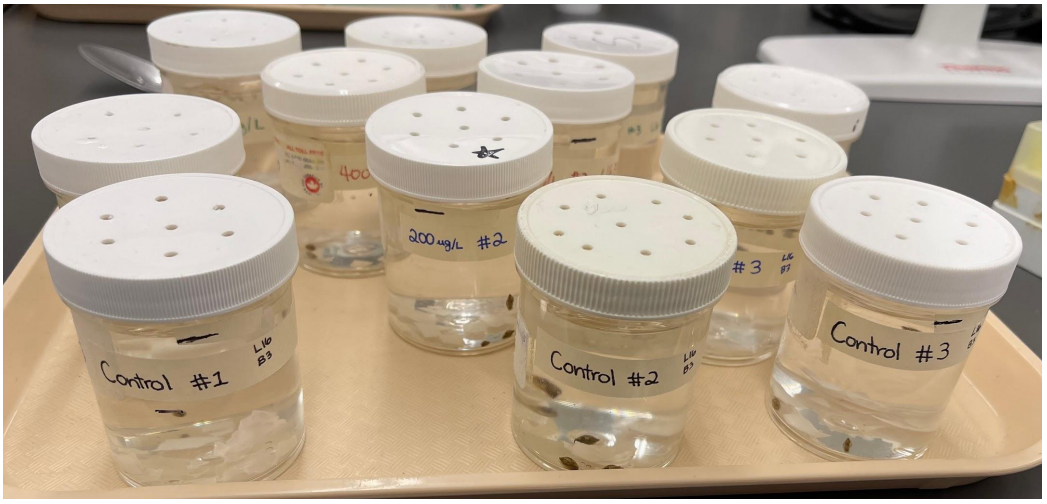

Figure 2. Bladder snails, *P. acuta*, in 12 experimental units for snail acclimation and toxicity test.

Four to five snails, following cluster sampling on age, were transferred from the bucket to 12 individual plastic containers filled with 100mL dechlorinated water and 0.5g of crushed egg shells for enriching the nutrients, such as calcium, in water (Figure 2). All units were kept in a 25ºC incubator for 24 hours. The egg shells were removed during the first water change.

Zinc toxicity tests

A zinc treatment stock solution (160 mg/L Zn) was prepared by dissolving zinc chloride into 50 mL of dechlorinated water. In the experimental units from the acclimation step, the stock solution was diluted to a 100mL of treatment solution with corresponding zinc concentrations: 200, 400, and 800 µg/L (Figure 2). The dilution was performed at the initiation of each treatment and after water changes per week.

Quality Control

Water change was performed every week, and 30% of the treatment solution in each replicate container was replaced. All experimental units were kept in a 25ºC incubator on a 12:12h light:dark photoperiod. Dissolved oxygen (DO) and pH levels were measured, using Vernier pH sensor and Traceable® Fisherbrand DO meter, after the initiation of treatment and before water change every week. Snails in the containers were provided with crushed algae wafers three times per week. The quantity (0.1~0.2 g) of algae wafers given in each container was determined by the number of live snails in each container.

Data Collection

Snail body mass (BM) and shell length (SL) were measured weekly since the start of zinc toxicity test. All surviving snails were weighed (±0.001 g) using a smart weigh JDS20 scale, and the shell length was measured (±0.002 cm) using a Vernier caliper. On the same day of the 30% water change, the numbers of surviving snails, dead snails, and egg masses were counted and egg masses were also categorized into three levels: small (10-15 eggs), medium (2-3 clusters), and large (≥4 clusters).

Statistical Analysis

Preliminary data of BM and SL was summarized to obtain mean mass/length and standard deviation (SD) for each replicate on every data collection day. The summary statistics were used to plot and determine the growth rate by finding the slope of the best-fit line. Three growth rates (g/day or cm/day) were obtained per variable of interest per treatment, and were summarized into mean growth rate with standard deviation for further analysis.

The BM and SL growth rates were assessed for the normality and homogeneity of variance by Shapiro-Wilk test and Bartlett's test in R (Hickey, 2019). The effect of zinc on BM and SL growth was evaluated with one-way analysis of variance (ANOVA), followed by a Tukey's Honestly Significant Differences (HSD) test (α=0.05) as post hoc using R (R Core Team, 2022).

Survival of *P. acuta* was quantified by dividing the final number of surviving snails by the initial number of snails for each replicate and expressing as a percentage (%) survival. Percentage survivals were analyzed qualitatively with descriptive statistics.

Results:

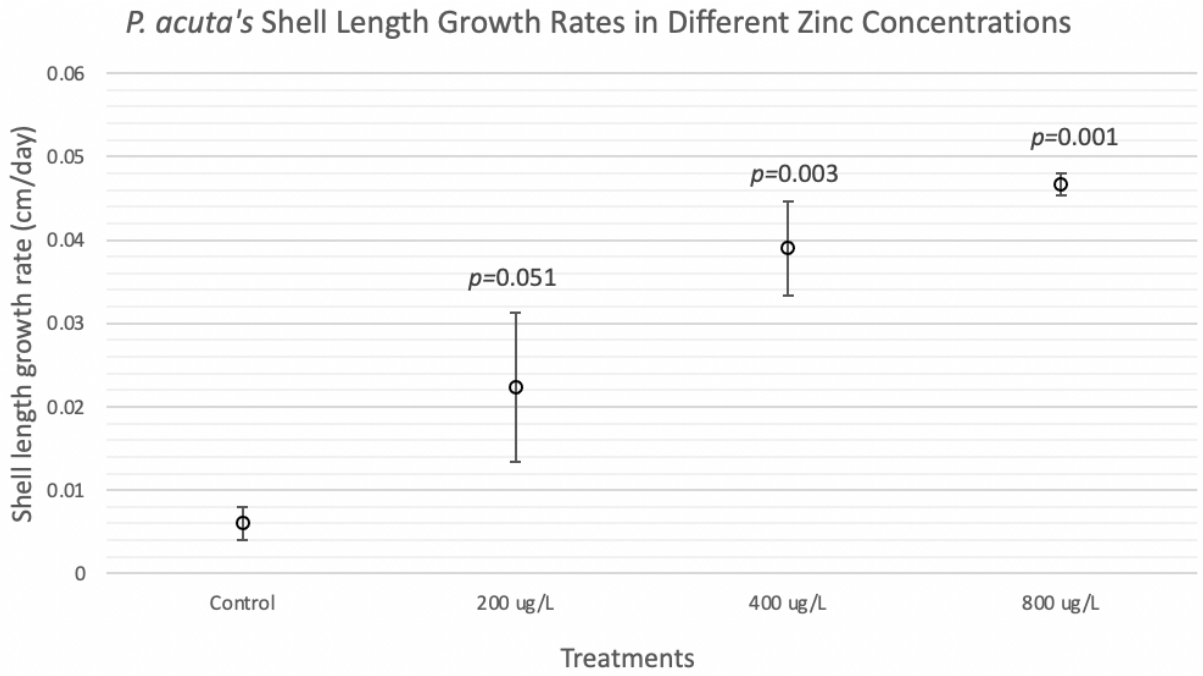

Figure 4. Growth rates (mean±SD) of shell length of *P. acuta* in different zinc concentrations. *p*-values indicated on chart were obtained by running Tukey's HSD against the control. ANOVA: $p<.001$

There is statistical significance in variations among all SL growth rates (ANOVA; $p < 0.001$). Figure 4 shows an increasing trend of the SL growth rate with increasing zinc concentrations. The growth rates of 800µg/L (0.0466±0.0013 cm/day), 400µg/L (0.0390±0.0056 cm/day), and 200µg/L (0.0223±0.0090 cm/day) treatments are 677%, 550%, 272% higher than that of the control (0.006±0.002 cm/day), respectively (Figure 4). By running Tukey's HSD, SL growth rates of control vs 800µg/L (p=0.001) and control vs 400µg/L (p=0.003) are significantly different (Figure 4).

Figure 5. Growth rates (mean±SD) of body mass of *P. acuta* in different zinc concentrations. *p*-values indicated on chart were obtained by running Tukey's HSD against the control. ANOVA: p=.015

One-way ANOVA reveals significance in variations among BM growth rates (ANOVA; p = 0.015). Figure 5 shows an increasing trend in the BM growth rate as the concentration of zinc increases, with a sudden drop of growth in the 400µg/L treatment (0.0615±0.0007 g/day). The growth rates of 800µg/L (0.095±0.0071 g/day), 400µg/L, and 200µg/L (0.071±0.0026 g/day) treatments are 93%, 25%, 44% higher than that of the control (0.0493±0.0168 g/day), respectively (Figure 5). By running Tukey's HSD, only the test of 800ug/L treatment against control is significant (p=0.01), while other pairwise comparisons are not (p > 0.05) (Figure 5).

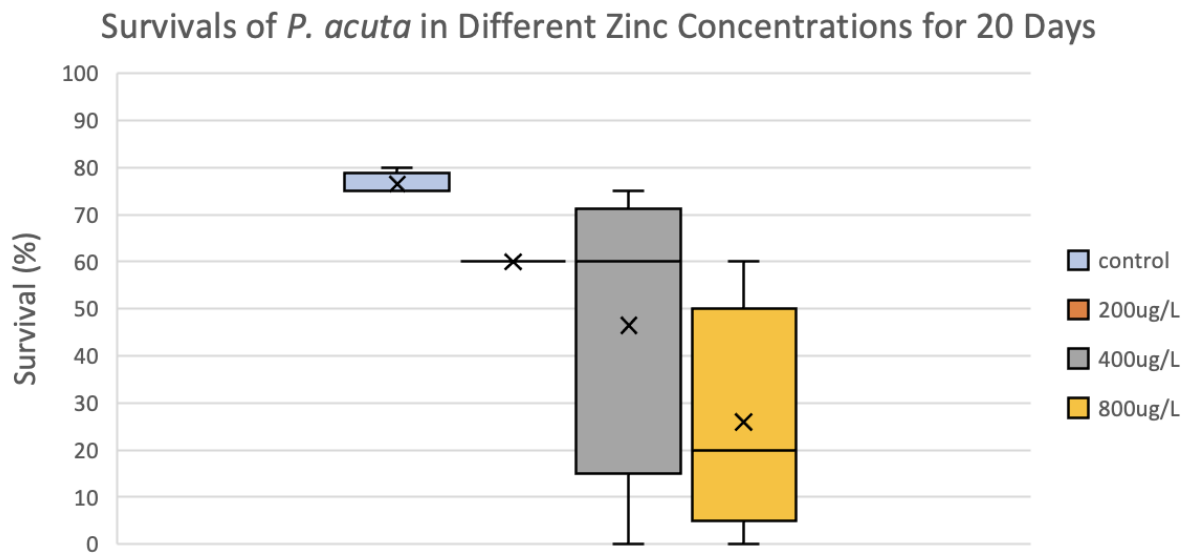

Figure 6. Summary boxplots for *P.acuta*'s survival under different zinc concentrations for 20 days. X-marker shows the mean, $\bar{S}$. Horizontal lines in a boxplot, from top to bottom, show max, 75th, 50th, 25th quartile, and min, respectively.

Throughout the experiment, the control (n_c=13) has the least number of dead snails (d_c=3), yielding a 76.67±2.88% survival, and the 800µg/L (n_{800}=14) has the most (d_{800}=10) (Figure 6). Overall, a decreasing mean percentage survival is shown with increasing zinc concentrations (Figure 6). The variation of percentage survival in control (75~80%) and 200µg/L treatment (60%) is narrow, while that of 400µg/L ($\bar{S}_{400}$=46.5%) and 800µg/L treatments ($\bar{S}_{800}$=26%) show a wider range from a minimum of 0% to a maximum of 75 or 60% survival (Figure 6).

One major observation on fecundity was that the fecundity of *P. acuta* decreased with increasing zinc concentration from 200µg/L treatment based on the total number of egg masses laid in the container for 20 days. The total count of egg mass in 200µg/L treatments had the highest count of egg mass (38) laid among other treatments, whereas the 800µg/L treatment had the least count of egg mass (13). The control had 28 small-sized egg masses laid, while the 400µg/L treatment had 21 egg masses in total. Seven and six medium-sized egg mass was found in both 200 and 400µg/L treatments respectively. Notably, one large-sized egg mass was only found in the 200µg/L treatments.

Discussion:

Our experiment demonstrated that increasing the zinc concentration had the shell length growth rate and body mass growth rate of *P. acuta* increased (Figure 4 and 5). The observed increase in growth rate based on the snail's shell length and body mass recorded contradicted our original hypothesis that predicted the negative

impact of increasing zinc concentration on growth. Previous research examining the effects of dietary exposure to different concentrations of zinc (0, 50, 100, 500, 1000, 10,000, and 15,000 µg per gram of dry food) on similar invasive (land) snail species, *Theba pisana,* revealed an inhibitory influence on their growth between the start and end of the five-week experiment (El-Gendy et al. 2011). Our investigation focused on concentrations 0, 200, 400, and 800 µg/L, not considering significantly higher concentrations of zinc as El-Gendy et al. conducted (2011). A possible reason for the contradiction is the ability of snails to tolerate zinc at lower concentrations. An example of these adaptive characteristics is the ability to bioaccumulate the metal in the shell, preventing the metabolic activation of the metal and the subsequent adverse effects on the mollusk tissue (Newman et al. 1994). Similar research based on this behavioural trait was conducted on other species of bladder snails, *Brotia costula*, and *Melanoides tuberculata,* which are comparable to our species of interest, *P. acuta,* similarly exhibited a preference for depositing zinc inside their shells, suppressing their metabolic effects on tissue (Lau et al. 1998). The bioaccumulation of zinc in snails may explain the increase in body mass growth rate in our study, where more zinc was deposited in the shell resulting in higher body mass, and thereby the rate, in more concentrated solution. Yet, our experiment does not examine these adaptations and, thus, requires further investigation into the bioaccumulation and physiology of *P. acuta* in response to zinc.

Although our results of growth rates are statistically significant, the small sample size for each replicate should be taken into consideration when reaching a conclusion. Growth rates are seen to increase as zinc concentrations increase (Figures 4 and 5), while survivability decreases (Figure 6). While not tested directly, there was an observable trend of decreasing fecundity with increasing zinc concentration based on the final number of egg masses laid. It is likely that the snails grown in lower zinc concentrations prioritized reproduction over individual growth. Overall, the increasing zinc concentration does not result in unfavourable growth effects on *P. acuta* but suggests a negative impact on their survival and fecundity.

One major source of uncertainty and variation in our experiment was during the acclimation stage and the first week of treatment. Initially, 0.5g of crushed egg shells and 0.5g of crushed algae wafers were added to each replicate. The water became cloudy with some replicates having a thin top layer of bacterial growth. This was due to high rates of bacterial decomposition indicated by the low dissolved oxygen concentrations. Since bladder snails have pallial lungs instead of gills, they are required to reach the surface of the water to breathe air. Many snails were not able to break through this film resulting in high mortality at this stage.

Conclusion:

The results showcasing a linear increase in shell length and body mass growth rates with treatment concentrations do not support our original predictions of zinc having a negative impact and leading to decreased shell length and body mass. The decreased mean percentage of survival may have occurred due to zinc indicating an impact to some degree. Despite these two major results, overall the varying concentrations of zinc in this experiment did not negatively impact *Physella acuta* as they were still able to thrive, reproduce with viable egg masses, and adapt to the varying levels of zinc.

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