REPRODUCTIVE LIFE HISTORY DECISIONS AND SUCCESS OF FRESHWATER POND SNAILS (*PHYSA ACUTA)* DURING CHRONIC ZINC EXPOSURE

By

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Abstract: Life history theory examines how individuals should make trade-offs between current reproductive effort and survival to achieve future reproductive gains. A prediction is that as future life expectancy decreases, individuals should invest more in current reproduction at the cost of lower survival and future reproduction. Although *P. acuta* have been shown to display a relatively high tolerance to anthropogenic contaminants and pollutants, research regarding sub-lethal chronic exposure to contaminants and reproductive effort is limited. However, it has been shown that higher zinc concentrations lower *P. acuta* survival rates. Therefore, the objective of this study was to determine if chronic sub-lethal exposure to zinc has the potential to alter an individual's reproductive life history decisions, and if the magnitudes of these decisions are dependent upon a site's historic zinc exposure. Snails were collected from 3 sites within the Grand Lake watershed that have different zinc concentrations. Their offspring were then exposed to one of 5 zinc concentrations over the course of ~18 weeks. Individuals from these sites showed differences in response to zinc treatments. Individuals from historically moderate zinc concentrations followed life history predictions most closely, as an increase in zinc treatment resulted in earlier timing of reproductive events and growth. However, individuals from historically low zinc exposure showed delayed growth and reproduction as zinc concentration increased. Individuals from high historic zinc exposure in general displayed few negative effects from the zinc treatments, likely due to a high zinc tolerance among these individuals. Overall, results showed evidence of a gradient of local adaptation and tolerance of zinc. Tolerance seemed to be a key factor in whether individuals make life history changes in response to metal contamination.

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CHAPTER I

INTRODUCTION

Grand Lake and Life History Theory

The Tri-State Lead-Zinc Mining District is a significant source of heavy metal contamination within the Grand Lake watershed. Studies have shown heightened levels of metals in the area, such as lead, zinc, and cadmium, within lake sediments (Burks and Wilhm 1995; Ingersoll et al. 2009, Hickey 2019, Morrison 2019). Although concentrations of these metals are high in some areas, the threat of critical or fatal toxicity to organisms residing within these contaminated locations remains low (Ingersoll et al. 2009). However, chronic exposure to this metal contamination could affect the life histories and reproductive success of resident organisms. Life history theory is an ecological framework used to better understand the diversity of survival and reproductive strategies, and why some strategies are utilized over others. In general, individuals make trade-offs between current reproductive effort and survival to achieve future reproductive gains (Krebs and Davies 1983). When adult future life expectancy is low, individuals will generally reproduce earlier and produce a greater number of offspring earlier in their lifetime (Stearns 1976). As the probability of future survival decreases, so does the number of potential reproductive events an individual may have in their lifetime. As individual survival is reduced, the window of time in which reproduction can occur shrinks. Therefore, to raise net reproductive fitness it is advantageous for individuals to invest more in reproductive efforts, thus reproducing earlier and with increased clutch sizes, but at a cost of decreased life expectancy. Therefore, it is hypothesized that in an unfavorable environment, an environment that decreases an individual's probability of survival, individuals will increase reproductive efforts in order to raise net reproductive fitness.

Metals Contamination and Freshwater Invertebrates

Although *Physa* typically display a moderately high tolerance to acute anthropogenic contaminants and pollutants (Goodnight 1973), studies have also shown many sub-lethal effects due to anthropogenic contaminant exposure in freshwater macroinvertebrates (Herricks & Buikema 1977, Kunce 2018). Studies have shown that a few heavy metals, for example, have a tendency to bioaccumulate in the soft body tissue of gastropods (Zadory 1984), which in turn can catalyze other physiological complications, such as inhibition of movement and growth (Lefcort et al 2013, Wadaan 2007). Heavy metals may also provide neurological challenges, as they have been shown to interfere with memory formation and kairomone reception in several freshwater invertebrate species (Byzitter et al 2012, Lefcort et al 2013). In addition to physical complications, heavy metals present behavioral difficulties as well. Inhibited kairomone reception due to heavy metal exposure has, for example, been linked to a decrease in predator avoidance responses and responses to conspecific odors in *Physa* and *Lymnaea* snails (Lefcort et al 2013).

Physa Reproduction

Although, the physical and behavioral responses of freshwater invertebrates, particularly Physa spp., have been studied in regards to heavy metal contamination, effects on life history and reproduction among freshwater macroinvertebrates have remained under-examined. The reproductive characteristics of the study species, *Physa acuta*, make them an ideal model system in regards to life history decisions. *Physa acuta* are a common freshwater gastropod found throughout much of the United States and the Grand Lake drainage. These snails are hermaphroditic and also have the ability to store sperm from a partner for long periods of time, in some cases up to sixty days or more (Wethington & Dillon 1991). These combined traits essentially allow the snails to reproduce whenever and in whatever environment they choose.

Objectives and Hypotheses

The objective of this study was to evaluate how *P. acuta's* reproductive life history decisions were affected by exposure to sub-lethal concentrations of zinc and by the site's historic exposure to zinc. Zinc, lead, and cadmium can regularly contaminate waterways and wetlands in close proximity to mining and agricultural operations and is abundant in portions of the Grand Lake region in northeastern Oklahoma. Because zinc, in particular, is present at high and frequently toxic concentrations in this region, it was chosen and used as the contaminant of interest for this study. Given that higher zinc concentrations lower future survival and decrease reproduction, I predicted the following responses:

1. *Length.* Across sites, individual shell length would increase as historic zinc concentration increased across sites. Across zinc treatments, I expected individual length to increase as zinc treatments increase.
2. *Egg Masses and Egg Production.* Across sites, I expected greater reproductive effort earlier in life as historic zinc concentration increased across sites. Reproductive effort is quantified in this study in terms of egg and egg mass production. I predicted that individuals would produce a higher proportion of their total eggs and egg masses earlier in their life as historic zinc concentration increased across sites. Across zinc treatments, I expected the same trend to occur.

3. *Hatching Success.* Across sites, I expected the hatching success of eggs to decrease as historic zinc concentration increased across sites. Across treatments, I expected hatching success of F_1 offspring to decrease among all F_1 individuals as F_1 treatment concentration increased.
4. *Effect of Tolerance.* While I expected all individuals to follow the predictions of life history theory, I also expected physical zinc tolerance to disrupt these predictions for individuals of very low zinc tolerance. I predicted that intolerant individuals originating from historically lower zinc concentrations than their treatment would be physically incapable to adhere to the predictions of life history theory due to the adverse effects of the zinc treatment itself. For intolerant individuals, I expected stunted growth, and consequently later reproductive maturity (Hickey 2019).

CHAPTER II

METHODS

General Design

I chose three sites of varying zinc concentrations within the Tar Creek Superfund site watershed: Rock Creek, Twin Bridges, and Tar Creek, henceforth referred to as the low zinc (LZ), medium zinc (MZ), and high zinc (HZ) sites respectively. I collected snails (F_0) on July 25, 2018 from each site, brought them back to the lab, and allowed them to reproduce in laboratory conditions in clean, dechlorinated water without the presence of zinc. I collected egg masses from the F_0 snails and allowed them to hatch. F_1 hatchlings emerged on approximately August 3, 2018. Ten days post-hatching, I exposed F_1 individuals to one of five zinc treatments: 0, 125, 250, 375, or 500 µg/L of zinc. Eight replicates were created for each site-by-treatment cross, resulting in a total of 120 experimental units (Figure 1). Each experimental unit consisted of one individual snail housed in 300ml of water within a plastic deli cup. F_1 water was changed twice per week, during which zinc treatments were reapplied and each snail received ~0.03g of Hikari brand algae wafers. F_1 individuals remained exposed to their treatments throughout the remainder of the experiment. I terminated the study on December 14, 2018, approximately 19 weeks after F_1 snails hatched. Thus, zinc treatments were administered for approximately 18 weeks.

Mating

On even weeks of the study, starting on week 4 until the termination of the study, each individual *Physa* was provided a mating opportunity. For each treatment snail, I counted and removed egg masses from the snail's cup, and then added three marked conspecifics to the cup. Each introduced conspecific was of the same age and originated from the same site as the focal snail, but was not a treatment snail. Before introduction, conspecifics' shells were painted with colored nail polish to distinguish them from focal individuals. I left the conspecific snails in the cups for two hours. I removed and disposed of any egg masses laid during the two-hour mating period, because I could not know which snail laid the eggs.

Length and Egg Masses

On odd weeks of the study, beginning week 3 until the termination of the study, I measured the aperture lengths of the snails using digital calipers. As soon as individuals reached reproductive age (~ 4 weeks), I counted the number of egg masses in an individual's cup. Three times per week, egg masses from each snail's cup were counted, and then removed from the cup. Egg masses that were not used in the hatching success analysis (discussed below) were disposed of.

Egg Counts and Hatching Success

At the beginning of each week, I collected a sample of egg masses from each individual to be counted and used to measure hatching success. 24 hours prior to sampling, cups were cleared of any current egg masses to ensure that all masses collected for the sample were no more than 24 hours apart in age. I placed each sampled egg mass under a dissecting counted the number of egg inside using a clicker. I then set aside the sampled egg masses for 10 days to allow them to hatch, and then counted successful hatchlings using a clicker. Since I was measuring hatching success, all hatchlings found outside of the egg mass were counted as successful, even if they appeared to no longer be alive.

Analyses

Egg Mass Production

I used egg mass production as a measure of reproductive decision making. I analyzed the weekly number of egg masses using negative binomial generalized mixed effects models (GLMMs) (Zuur et al. 2010). Models were dredged using the following model in order to ensure every possible model combination was present:

$$Number\ of\ egg\ masses \sim Age + Age^2 + Concentration + Site + Age{:}Concentration + Age{:}Site + Age^2{:}Concentration + Age^2{:}Site + Concentration{:}Site + Age{:}Concentration{:}Site + Age^2{:}Concentration{:}Site + (1|ID))$$

Descriptions of model terms are displayed in Table 1. To compare these models, I used Akaike's Information Criterion (Burnham & Anderson 2002) with an adjustment for relatively small sample sizes (AICc) within the *bblme* package in RStudio to quantify the evidence for each of the alternative models from the data. The best supported model was used to make data predictions by utilizing the “predict” function in RStudio. Model predictions were created for expected number of egg masses to be produced each week across all weeks of the study in which individuals were reproductive (weeks 4-17).

Egg Production

Egg production measures were used as a measure of age-specific snail fertility. Egg counts taken within the same 24-hour period each week were used as a measure of weekly egg production. I used the same methods as the egg mass analysis to make prediction models describing egg production. Model predictions were created to estimate the expected hatching success rate of each site-by-treatment combination across all weeks on the study in which individual egg counts were conducted (weeks 5-17).

To determine whether the timing of egg production was shaped directly by zinc concentrations or indirectly by zinc effects on snail size, weekly egg count data was then analyzed by creating models that used treatment and size as explanatory variables. Since length measurements were taken biweekly, and egg count data was collected weekly, only egg count data that corresponded to the biweekly length data was used in this analysis. I created alternative models examining whether egg production was explained by snail length and zinc treatment. Analyses were done separately for the three sites using AIC.

Hatching Success

Hatching success data was analyzed as binomial data within generalized mixed effects models (GLMMs). I used the same methods as the egg mass analysis to create hatching success prediction models. Model predictions were created to predict the expected hatching success rate of each site-by-treatment combination across all weeks on the study in which individuals were reproductive (weeks 4-17).

Age of First Reproduction and Survival

First reproduction events and mortality events were analyzed by performing a survival analysis in RStudio using the *survival* package. To examine timing of reproductive events, I created survival models using a logistic distribution. To examine timing of reproductive events, I created survival models using a logistic distribution. To compare these models, I used the AIC function within the *bblme* package in R Studio. To examine the timing of individual deaths over the course of the study, I created survival models using a Gaussian distribution.

CHAPTER III

RESULTS

Length

Across sites, average shell length over the course of the study did not differ drastically within each control group (Figure 2A). The most substantial increase in length occurred between weeks 4 and 8. Within the MZ and HZ sites (Figures 2C & 2D), shell length generally seemed to increase as concentration increased, however, differences between treatments did not seem to be consistent. Conversely, individuals from LZ displayed the opposite trend, with shell length decreasing as zinc concentration increased (Figure 2B).

The best supported model had length depending on age, age squared, zinc concentration, site, concentration pairwise interacting with age, age squared, and site, age and site interacting, and the three-way interaction of age, concentration, and site (Table 2). Based upon this best supported model, HZ individuals were predicted to have the longest shell length of the three sites, regardless of week (Figure 3D). Shell length predictions did not seem to differ between LZ and MZ sites (Figure 3B & 3C). The most substantial increase in length was predicted to occur between weeks 4 and 9. The model showed that as zinc concentration increased shell length increased in MZ and HZ sites and decreased in the LZ site.

Weekly Egg Mass Production

For each control group, egg mass production typically peaked at ~7-8 weeks after which point egg mass production began to steadily decline (Figure 4A). Individuals originating from HZ generally produced more egg masses per week on average than MZ and LZ individuals across control groups. Among LZ individuals, egg mass production peaked between weeks 7-9 when individuals were exposed to lower zinc concentrations (0, 125, and 250μg/L zinc), while egg mass production peaked between weeks 10-12 during higher concentration zinc treatments. MZ and HZ individuals generally displayed higher egg mass production as zinc concentration increased.

Based upon the AIC comparison of the egg mass production models, the model best supported by the data (Table 3) had egg mass number depending on age, age squared, zinc concentration, site, site pairwise interacting with age, age squared and concentration, age interacting with concentration, and the three-way interaction of age, concentration, and site. Based upon this best supported model, egg mass production predictions among control individuals indicated that those originating from HZ and LZ were predicted to peak in egg mass production at 9 weeks of age, approximately 3-4 weeks before individuals originating from the MZ site (Figure 5A).

Among LZ individuals, snails were predicted to produce less egg masses per week as zinc concentration increased. Additionally, across all treatments among LZ individuals, peak egg mass production seemed to be delayed as zinc treatment increased. MZ individuals were predicted to peak in egg mass production between weeks 10 and 12. MZ individuals also displayed the opposite trend in egg mass production across treatments, as there was an increase in egg mass production as zinc concentration increased. HZ individuals also showed an increase in egg mass production as the zinc levels were increased. Among HZ snails, egg mass production peaked at approximately 10 weeks across all treatments.

Lifetime Egg Mass Production

Upon analyzing total lifetime egg mass production using AICc, the model containing Site and Concentration received the most support from the data (Table 4). The model containing Site alone as an explanatory variable was also well supported. For the top model, site and concentration were both shown to have a positive effect on total egg production for LZ and HZ individuals (Table 5). Among MZ individuals, concentration was shown to have a positive effect, while site displayed a negative effect. The model containing site alone showed similar effects of site on total egg masses.

Egg Production

Overall, egg production decreased with age across all individuals. LZ individuals generally showed similar egg production across the 0, 375, and 500µg/L zinc treatments, while the 125 and 250µg/L zinc treatments showed significantly higher egg production around weeks 7-9 (Figure 6B). Among HZ individuals, egg production seemed to increase as the treatment concentration increased (Figure 6D). There was little difference in egg production overall across treatments among MZ individuals (Figure 6C). Egg production did not seem to differ among control individuals (Figure 6A).

The model receiving the most support had egg production depending on age, age squared, zinc concentration, site, concentration pairwise interacting with age, age squared, and site, age and site interacting, and the three-way interaction of age, concentration, and site (Table 6). For LZ individuals, egg production decreased as zinc concentration increased (Figure 7B). However, for MZ and HZ individuals egg production increased as zinc concentration increased (Figure 7C & 7D). HZ individuals were predicted to produce the most eggs overall.

Analyses that used treatment and length as explanatory variables for egg production showed that for the LZ, egg production was best explained by snail length and zinc concentrations (Table 7). For MZ and HZ site, the best explanation also included the interaction between length and concentration. For all of the sites, the supported models showed a positive effect of zinc concentration and snail length on egg production (Table 8). However, a larger effect size was found for length in LZ individuals, while concentration was shown to have a larger positive effect size in MZ and HZ models.

Hatching Success

For each control group, there did not appear to be a significant difference in hatching success rate across the three sites (Figure 8A). Among LZ individuals, there did seem to be a significant difference in hatching success across treatment groups (Figure 8B). Hatching success remained constant and relatively high for individuals receiving the 375 and 500µg/L zinc treatments. However, individuals who received the 0, 125, and 250µg/L treatments saw a decline in offspring hatching success over the course of the study.

The full model received the most support from the data (Table 9). Control individuals from HZ and LZ were both predicted to show a decline in hatching success throughout their lifetime, while MZ individuals' hatching success was predicted to increase over time by ~20% (Figure 9A).

LZ individuals displayed a positive trend in hatching success as zinc concentration increased (Figure 9B). This trend intensified with age. The opposite trends were displayed among HZ individuals, as percent hatching success was shown to decrease as zinc concentration increased (Figure 9D). MZ individuals displayed some fluctuation in hatching success over time.

Among MZ individuals, hatching success of offspring was predicted to increased towards the end of their life when individuals received a low zinc treatment, and a decreased hatching success when higher zinc treatments were received (Figure 9C).

Age at First Reproduction and Survival

Upon comparison of the first reproduction models, the full model, which contained a site-by-concentration interaction, was best supported by the data (Table 10).

During control treatments, individuals from the HZ and MZ sites appeared to start reproduction earlier than LZ individuals (Figure 10A). Among LZ individuals, the time of first reproduction occurred earlier for individuals in lower concentration zinc treatments (Figure 10B). Additionally, lower concentration zinc treatments (0, 125, and 250µg/L zinc) obtained higher proportions of reproductive individuals, than higher zinc treatments, and achieved these higher proportions earlier in life. MZ individuals however showed the opposite trend. As zinc concentrations increased, individuals generally reproduced earlier and attained higher proportions of reproductive individuals (Figure 10C). HZ individuals generally completed their first reproductive event at approximately 5 weeks of age, regardless of treatment. The proportion of reproductive individuals increased at approximately the same rate across all treatment groups among HZ snails (Figure 10D).

Over the course of the 18-week study, 37 deaths occurred which accounted for roughly ~30% of the focal individuals (N=120). After performing a survival analysis on survival models, the null model was best supported by the data (Table 11).

CHAPTER IV

DISCUSSION

Length

The zinc treatment ultimately seemed to have an impact within sites, but not necessarily across sites. I predicted that individual shell length would increase as historic zinc concentration increased across sites with the logic that individuals from those sites would be locally adapted for a quicker lifecycle to withstand the accumulated adverse effects of the zinc contamination. I found this prediction to be unsupported by the data. Average length did not seem to differ much across control groups (Figure 2A). Model predictions also showed no difference between LZ and HZ control individuals in regards to average weekly shell length (Figure 3A).

Across zinc treatments, I expected individual length to increase as zinc treatments increased. This prediction only seemed to be supported within the HZ site, since average shell for these individuals seemed to increase as zinc concentration increased (Figure 2D). However, this trend did not appear in MZ and LZ sites (Figure 2B & 2C), likely due to the ill physical effects of the zinc treatments, such as reduced growth rate (Wadaan 2007). LZ and MZ individuals' have been exposed to a lower historic zinc exposure in comparison to HZ individuals, likely resulting in a lower zinc tolerance among individuals of these sites (Hickey 2019). This low zinc tolerance may explain the exaggerated difference in individual length across LZ zinc treatments in comparison to MZ individuals, since LZ individuals historically have the least zinc exposure of the three sites.

Zinc treatment did not seem to have an adverse effect on average length among HZ individuals, since average weekly shell length increased as zinc treatment increased among HZ individuals. This trend persisted in the length model predictions for HZ individuals as well as MZ individuals, though was more exaggerated in the HZ site (Figure 3C & 3D). This lack of zinc aversion among HZ individuals may be attributed to the site's high historic zinc exposure, and consequent high tolerance of zinc (Hickey 2019). High historic zinc exposure within the Tar Creek watershed may have prompted individuals to make necessary life history decisions in these unfavorable environments to increase survival (Krebs and Davies 1983), such as increasing investment in growth, rather than reproduction to increase current fitness (Reznick 1983). Furthermore, in some studies, tolerant individuals in fact fair better in a moderately contaminated environment, rather than a completely clean environment due to the unnecessary energy expenditure of regulatory or excretion mechanisms operating in a clean environment (Hamilton et al. 2017, Hickey 2019).

Egg Masses and Egg Production

Overall, reproductive timing across and within sites did not seem to adhere to life history predictions. Across sites, I expected greater reproductive effort earlier in life in individuals from sites with greater historic zinc concentrations. This hypothesis did not appear to be supported. Across all sites’ control groups, the timing of peak egg mass and egg production did not seem to differ greatly (Figure 4A & 6A). However, model predictions did suggest that MZ individuals would peak in egg mass production approximately 3 weeks later than individuals form the LZ and HZ sites (Figure 5A).

I also predicted that across zinc treatments, greater reproductive effort would occur earlier in an individual's life as zinc concentration increased. This hypothesis also seemed unsupported. Interestingly, LZ individuals seemed to show the reverse of this prediction, as peak egg mass and egg production seemed to become increasingly delayed as zinc treatment increased (Figure 4B and 6B). This is likely attributed to a delay in reproductive maturity due to slowed growth instigated by exposure to the zinc treatments (Wadaan 2007). These results suggest that among LZ individuals, zinc treatment had an indirect, rather than direct effect on the timing of egg production via a physical delay in reproductive maturity. This delay in maturity seemed to be absent in the MZ and HZ sites, which is likely due an increased zinc tolerance among individuals from these sites (Hickey 2019).

The age at first reproduction analysis most clearly showed how zinc tolerance and life history decisions within individual sites affected the timing of reproduction within these groups. MZ individuals seemed to match life history predictions in regards to the timing of reproduction (Figure 10C). As zinc treatment increased, MZ individuals reproduced earlier in their lifetime. Since zinc has been shown to have a negative impact on survival rate (Hickey 2019), individual survival probability likely decreased as zinc treatment increased. Following the assumptions of life history theory, as adult survival decreases, individuals reproduce earlier (Stearns 1976). Although MZ individuals seemed to follow these predictions, LZ and HZ individuals did not. LZ individuals seemed to show the opposite trend; as zinc treatment increased across this site, reproduction was increasingly delayed (Figure 10B). This is likely due the low zinc tolerance of individuals from this site (Hickey 2019). Individuals were likely unable to follow these assumptions due to the direct adverse physical effects of the zinc treatment, most notably a delay in growth (Figure 3B), and consequently, a delay in reproductive maturity. Conversely, HZ individuals did not differ between treatments in regards to timing of reproduction (Figure 10D). Because HZ individuals have a high zinc tolerance, the concentrations of zinc treatments used in this study presumably did not cause HZ individuals enough stress to produce a difference in survival rates across treatment groups.

Although the timing of reproductive events over an individual's lifetime did not differ greatly across and within sites, the quantity of offspring produced did seem to be affected by the zinc treatment. The number of egg masses and eggs that were produced varied across zinc treatment groups within each site. This could be an indication of the utilization of different life history strategies regarding the optimal number of offspring to produce in different environmental conditions. LZ individuals were predicted to reproduce more overall as zinc concentration decreased (Figure 5B & 7B). This trend in offspring quantity among LZ individuals seemed to coincide with David Lack's clutch size principle (Lack 1954), which suggests that parents will produce as many offspring as the parent environment will allow them to produce. If zinc treatment has a negative effect on the health and fitness of LZ individuals, these high zinc environments would limit the amount of offspring that parent snails are able to produce. However, MZ and HZ snails seemed to exhibit the opposite trend, since treatment seemed to have a positive effect on reproductive efforts. If the snails from these sites are indeed more tolerant of zinc, these high zinc environments may not limit, or may at least lessen the limitations, on the amount of offspring the parent snails are able to produce.

Hatching Success

Across sites, I predicted that the hatching success of F_1 offspring would decrease as historic zinc concentration increased across sites. However, there did not seem to be a noticeable difference in offspring hatching success across control groups. Hatching success data seemed to be relatively unstable over the course of the 14-week period (Figure 8). This instability may be attributed to a low sample size and potential for human error throughout this particular data collection. Additionally, egg masses with fewer eggs yielded disproportionately more stochastic results, due to the binomial nature of the data. This stochasticity may have also played a role in the instability of the data, especially data collected towards the end of the study, when individuals were laying less eggs overall.

In addition, I also predicted that hatching success would decrease as treatment zinc concentration increased. Among MZ and HZ sites, there did not appear to be a clear trend in hatching success of offspring related to the zinc treatment (Figure 8C & 8D). However, upon examination of the top statistical model, zinc treatment seemed to have a positive effect on hatching success early in life, and a negative effect as individuals aged (Figure 9C & 9D). However, among LZ individuals, the zinc treatment interestingly seemed to positively affect offspring hatching success (Figure 9B). This result could be due to reduced egg production in higher zinc treatments among individuals form the LZ site. Alternatively, higher hatching success from higher zinc treatment groups could indicate a reproductive decision to produce higher quality offspring, rather than a higher quantity of offspring in an unfavorable environment (Parker & Begon 1986, Fischer et al. 2011, Taborsky 2006). Parker and Begon (1986) suggested that if mothers can anticipate their offspring’s future environment, they can adjust their offspring investment accordingly. In an unfavorable environment, larger offspring are typically associated with higher survival rates (Taborsky 2006). However, larger, higher quality, offspring often mean fewer offspring (Parker & Begon 1986). LZ individuals may have altered their reproductive decisions regarding the quality of their offspring due to the quality of their current environment.

Alternatively, healthier, or more tolerant, LZ individuals may have been disproportionately alive towards the end of the study. This could produce a disproportionately high survival rate of offspring, if heartier LZ individuals are likely to produce heartier offspring. Future studies might investigate the hatching success of egg masses exposed directly to zinc treatments to fully measure the effect of zinc on offspring survivability.

Conclusions

This study has provided insights into how heavy metals toxicity and the tolerance of toxicity may impact the life history decisions of aquatic fauna. Zinc treatments mainly appeared to show site-specific effects on *Physa* growth and reproduction. That is, sites displayed a relatively unique trends regarding growth and reproduction in response to zinc. Maturity and timing of peak reproductive activity seemed to be increasingly delayed in LZ individuals as zinc treatment increased. Quantitatively, LZ individuals grew larger and produced more offspring overall as zinc treatment decreased. Alternatively, HZ and MZ individuals showed no delay in maturity or timing of peak reproductive activity when exposed to the zinc treatments. Additionally, these MZ and HZ individuals grew larger and produced more offspring overall as zinc concentration increased. These site-specific differences are likely attributed to site-specific zinc tolerances. However, other possible site-specific factors may have played a role in the development of different life history patterns, such as the type of body of water (i.e. a flowing stream or stagnant lake) or the amount of predation risk in the area. Additionally, individuals with tolerance to high zinc concentrations may also have lower tolerance to low zinc concentrations (Hamilton et al. 2017). Utilizing a wider range of concentrations and environmental factors to measure life history and toxicity responses is necessary in order to more fully grasp the implications of these responses.

Knowing how different environmental changes and resilience can affect life history decision making within and across geographical locations can give us a more complete perspective into population dynamics and ecological change. Although snails within the Grand Lake watershed are subjected to physiological stressors (Hickey 2019), this study has provided evidence to support that life histories of individuals from these sites are also altered by chronic exposure to heavy metals. However, due to the opposing effects of zinc exposure and zinc tolerance, these changes in life history may be complex. For individuals with low tolerance of zinc, the presence of a high concentration of zinc may physically affect the snail to such a degree that they are unable to increase reproductive effort. At the other end of the spectrum, an individual with high zinc tolerance might not experience much stress from elevated zinc levels, and would thus have no reason to alter their reproductive decision making. Only when the negative effects of zinc exposure and the positive effects of zinc tolerance reach a rough equilibrium does it seem that life history predictions fully take effect. Awareness of these opposing forces and their effects on ecological behavior have the potential to aid in the predictability of ecological and evolutionary change within and across populations.

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APPENDICIES

Table 1. Model terms and descriptions.

Model Term	**Variable Type**	**Description**
Age	Independent	Age of focal snail (weeks)
Age^2	Independent	Age squared
Concentration	Independent	Concentration of zinc treatment
Site	Independent	Collection site for F_0 snails
ID	Random Effect	Unique identification of individual focal snails
Length	Dependent	Total shell length, measured bi-weekly
Number of Egg Masses	Dependent	Number of egg masses produced per individual, measured weekly
Number of Eggs	Dependent	Number of eggs produced within a single egg mass, measured weekly
Hatching Success	Dependent	Successful hatchlings/eggs counted within an egg mass, measured weekly

Table 2. Alternative models for snail length. All possible model combinations were compared. Models containing ∆AICc scores of lower than 7.0, the full model, and null model are displayed.

Model	**∆AICc**	**df**
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Concentration + Concentration:Site + Age:Concentration:Site	0.0	16
Age + Age^2 + Concentration + Site + Age:Concentration Age^2:Concentration + Age^2:Site + Concentration:Site + Age^2:Concentration:Site	2.5	16
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Concentration + Concentration:Site	2.8	14
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Concentration + Age^2:Site + Concentration:Site + Age^2:Concentration:Site	3.0	18
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age2:Concentration + Age^2:Site + Concentration:Site + Age:Concentration:Site	3.2	18
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Concentration + Age^2:Site + Concentration:Site + Age:Concentration:Site + Age^2:Concentration:Site (Full model)	5.2	20
Age + Age^2 + Concentration + Site + Age:Concentration + Age^2:Concentration + Age^2:Site + Concentration:Site	5.5	14
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Concentration + Age^2:Site + Concentration:Site	6.0	16
Null	1135.2	3

Table 3. Alternative models for weekly egg mass production All possible model combinations were compared. Models containing ∆AICc scores of lower than 7.0, the full model, and null model are displayed.

Model	**∆AICc**	**df**
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Site + Concentration:Site + Age:Concentration:Site	0.0	17
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Concentration + Age^2:Site + Concentration:Site + Age:Concentration:Site + Age^2:Concentration:Site (Full model)	0.2	20
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Site + Concentration:Site + Age:Concentration:Site	0.7	18
Age + Concentration + Site + Age:Concentration + Age:Site + Concentration:Site + Age:Concentration:Site	3.0	15
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Concentration:Site + Age:Concentration:Site	4.0	16
Null	462.1	3

Table 4. Alternative models describing total lifetime egg mass production. All models are shown.

Model	**ΔAICc**	**df**
Site+Concentration	0.0	5
Site	0.7	4
Site*Concentration (Full)	1.6	7
Concentration	8.4	3
Null	9.2	2

Table 5. Lifetime egg production top model summary. Site effects are relative to the LZ site. Thus, if zinc concentration was 0, expected egg production would be 45.9 and 67.3 for the MZ and HZ sites respectively.

Variables	**Estimate**	**Std. Error**
Site (LZ) (Intercept)	49.4	4.4
Site (MZ)	-3.5	6.3
Site (HZ)	17.9	6.3
Concentration	4.4	2.5

Table 6. Alternative models for egg production. All possible model combinations were compared. Models containing ΔAICc scores of lower than 7.0, the full model, and null model are displayed.

Model	**ΔAICc**	**df**
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Concentration + Concentration:Site + Age:Concentration:Site	0.0	16
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Concentration:Site + Age:Concentration:Site	1.6	15
Age + Age^2 + Concentration + Site + Age:Concentration + Age^2:Concentration + Age^2:Site + Concentration:Site + Age^2:Concentration:Site	2.5	16
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Concentration + Age^2:Site + Concentration:Site + Age:Concentration:Site	3.2	18
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Concentration + Age^2:Site + Concentration:Site + Age^2:Concentration:Site	3.7	18
Age + Age^2 + Concentration + Site + Age^2:Concentration + Age^2:Site + Concentration:Site + Age^2:Concentration:Site	4.1	15
Age + Age^2 + Concentration + Site + + Age:Concentration + Age:Site + Age^2:Site + Concentration:Site + Age:Concentration:Site	4.8	17
Age + Age^2 + Concentration + Site + Age:Concentration + Age^2:Concentration + Age^2:Site + Concentration:Site + Age^2:Concentration:Site	5.4	17
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Concentration + Age^2:Site + Concentration:Site + Age:Concentration:Site + Age^2:Concentration:Site (Full model)	7.0	20
Null	174.5	3

Table 7. Models describing effects of size and treatment concentration on egg production. Each site was analyzed separately. All models are shown.

	LZ		**MZ**		**HZ**	
Model	**ΔAICc**	**df**	**ΔAICc**	**df**	**ΔAICc**	**df**
Null	30.0	3	28.4	3	13.0	3
Concentration	27.3	4	25.8	4	4.1	4
Length	1.6	4	1.8	4	8.6	4
Concentration+Length	0.0	5	0.5	5	1.3	5
Concentration*Length (Full model)	0.4	6	0.0	6	0.0	6

Table 8. Summaries of the size and zinc treatment effects on egg production according to the top model. Bolded values indicate y-intercept value.

	LZ		**MZ**		**HZ**	
Variable	**Estimate**	**Std. Error**	**Estimate**	**Std. Error**	**Estimate**	**Std. Error**
Concentration	1.0	2.3	7.5	8.2	20.1	14.5
Length	4.7	0.8	4.5	0.9	2.3	1.3
Concentration+Length	**-19.9**	7.1	**-19.9**	8.5	**5.5**	14.3
Concentration*Length	-	-	-0.9	0.9	-1.3	1.4

Table 9. Alternative models describing hatching success rates. All possible model combinations were compared. Models containing ΔAICc scores of lower than 7.0, the full model, and null model are displayed.

Model	**ΔAICc**	**df**
Age + Age^2 + Concentration + Site + Age:Concentration + Age:Site + Age^2:Concentration + Age^2:Site + Concentration:Site + Age:Concentration:Site + Age^2:Concentration:Site (Full model)	0.0	19
Null	1127.0	5

Table 10. ΔAIC scores of models describing age of first reproduction. All models are shown.

Model	**ΔAIC**	**df**
Site:Concentration (Full model)	0.0	7
Site	9.6	4
Site+Concentration	11.6	5
Null	16.9	2
Concentration	18.8	3

Table 11. ΔAIC scores of mortality models. All models are shown.

Model	**ΔAIC**	**df**
Null	0.0	2
Concentration	2.0	3
Site	3.7	4
Site+Concentration	5.7	5
Site:Concentration (Full model)	9.0	7

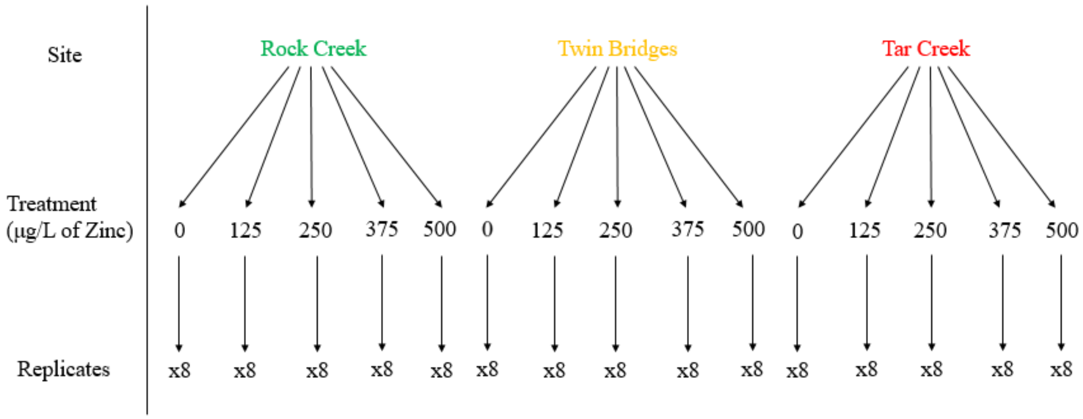

Figure 1. Experimental Design.

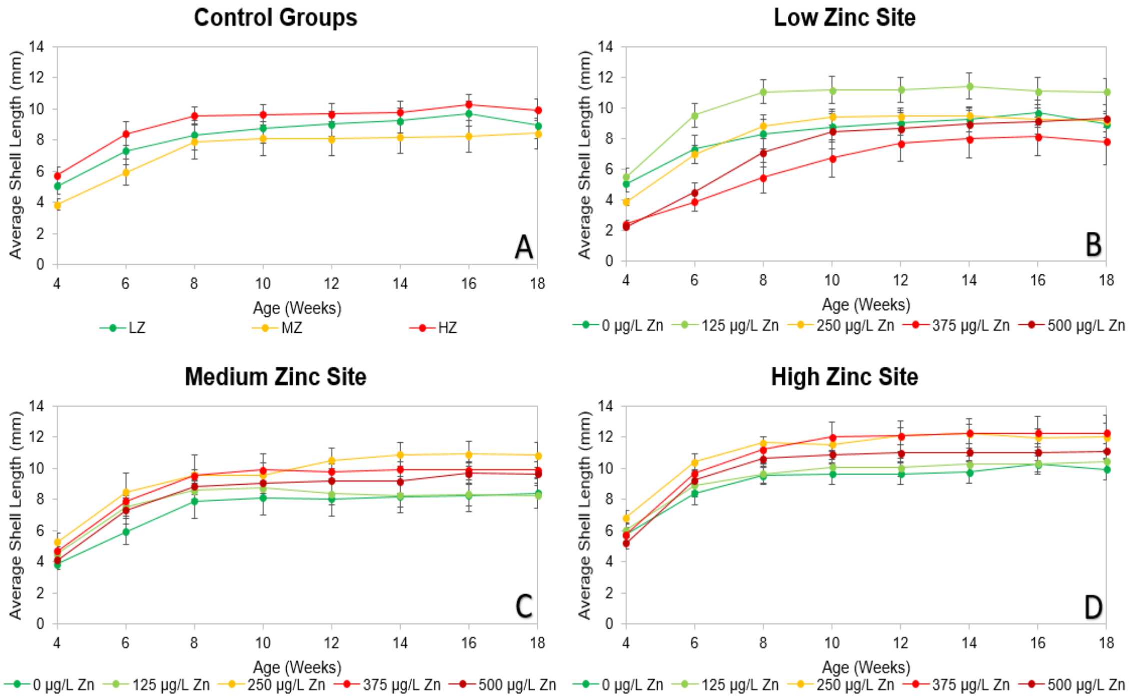

Figure 2. Average shell length (aperture to spire) of experimental individuals. Error bars indicate standard error.

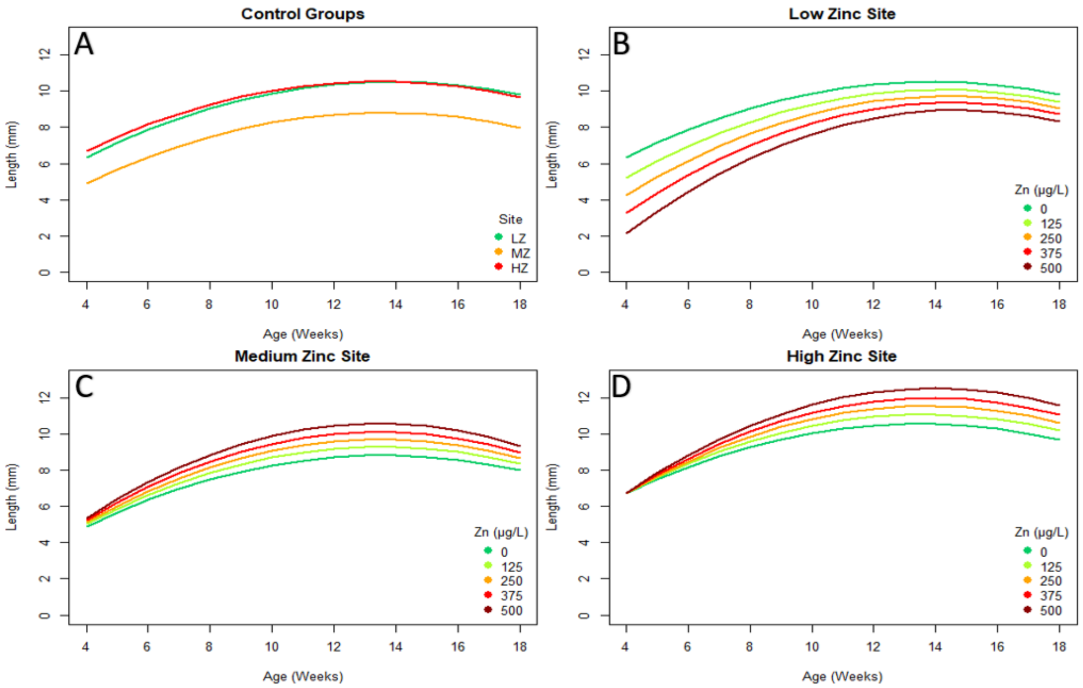

Figure 3. Weekly shell length as predicted from the best supported model.

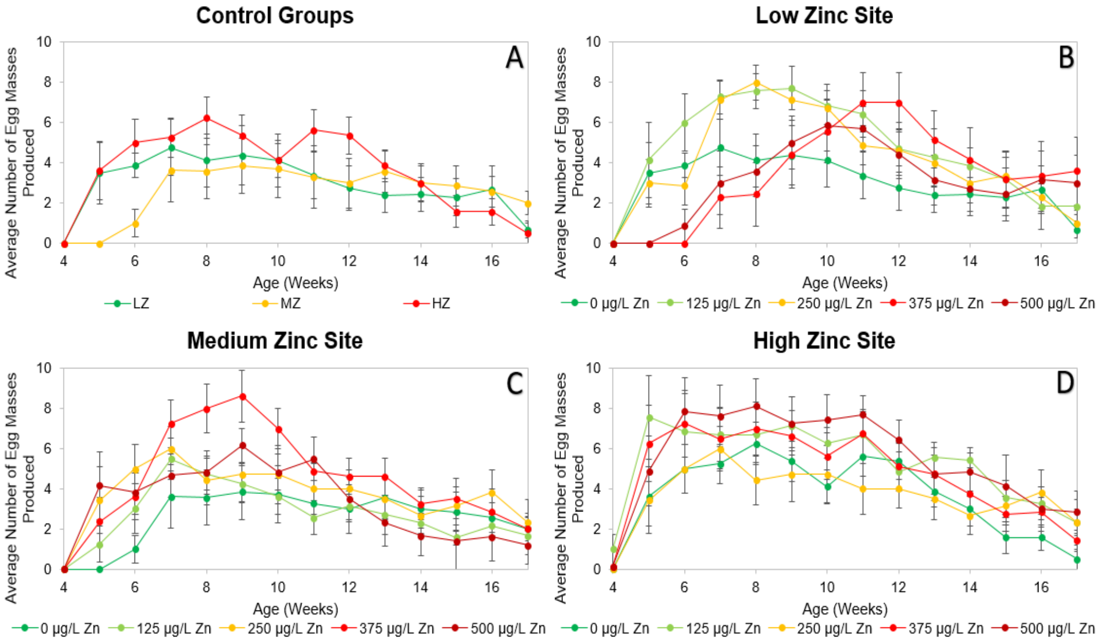

Figure 4. Average number of egg masses produced per week. Error bars indicate standard error.

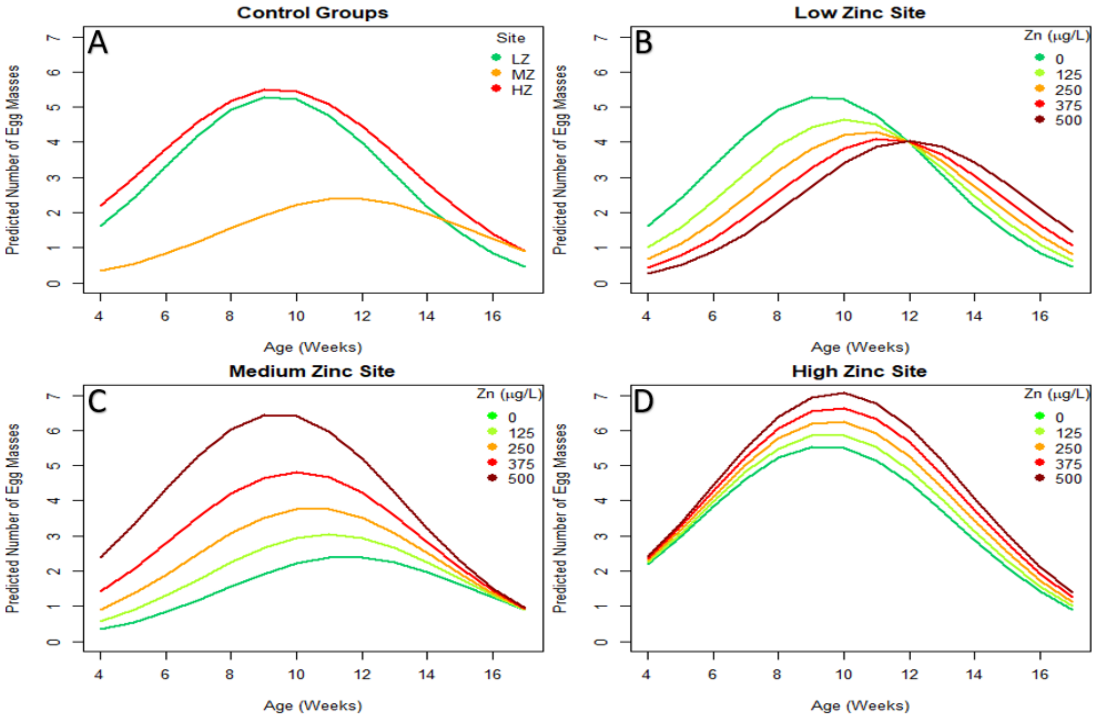

Figure 5. Predicted number of egg masses produced per week.

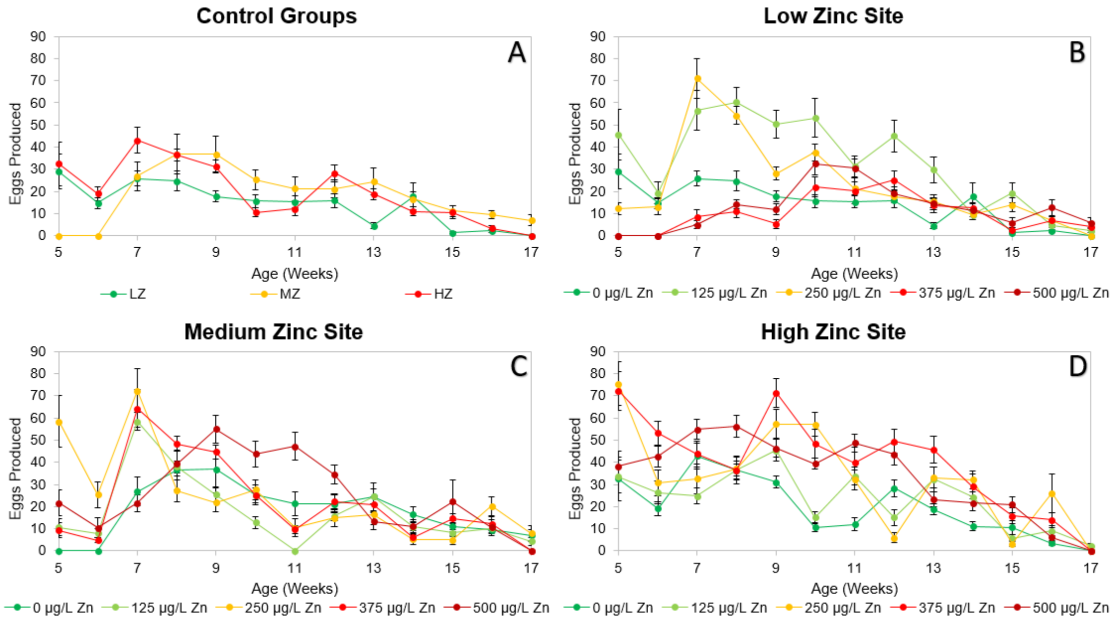

Figure 6. Number of eggs produced over a 24-hour time span each week. Error bars indicate standard error.

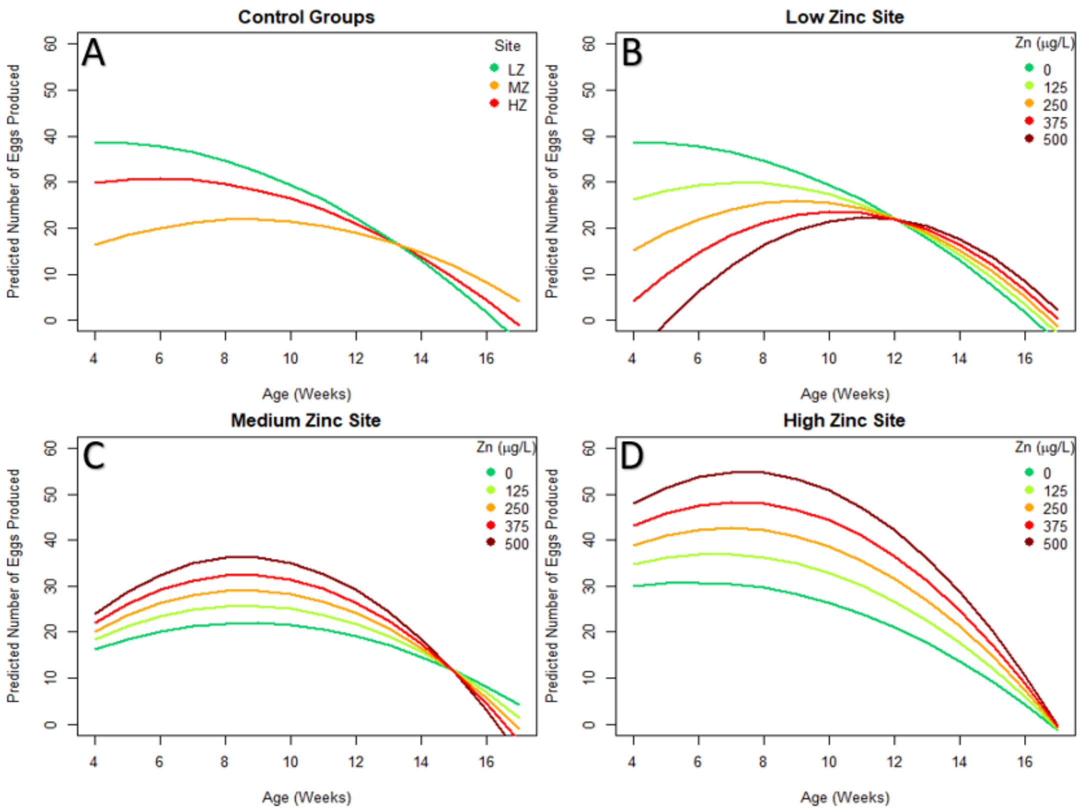

Figure 7. Predicted number of eggs to be produced within 24-hour time span by age (weeks).

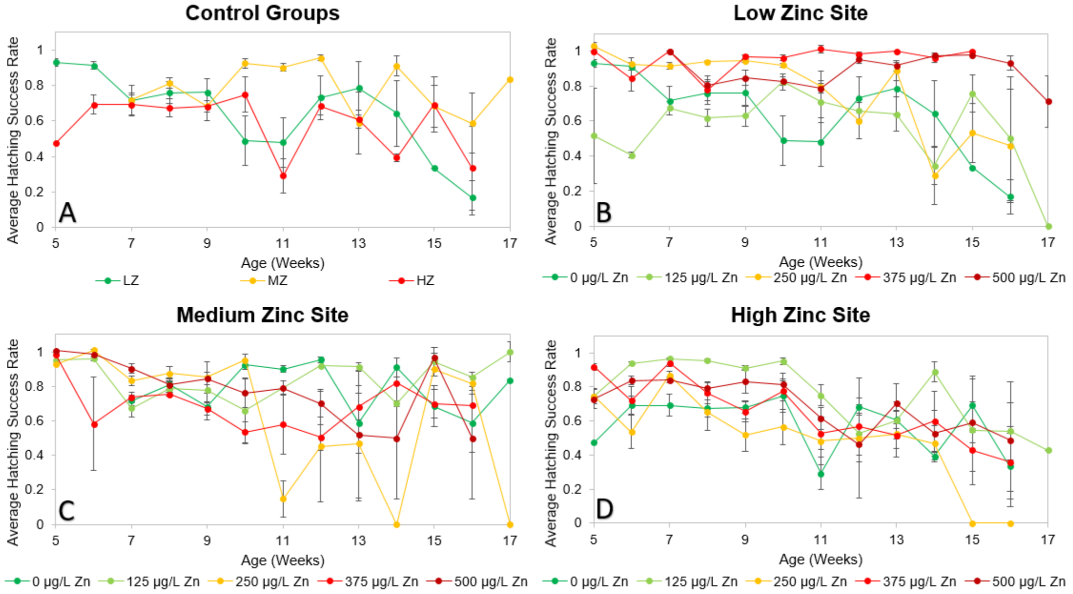

Figure 8. Average hatching success rates of F_1 offspring. Error bars indicate standard error.

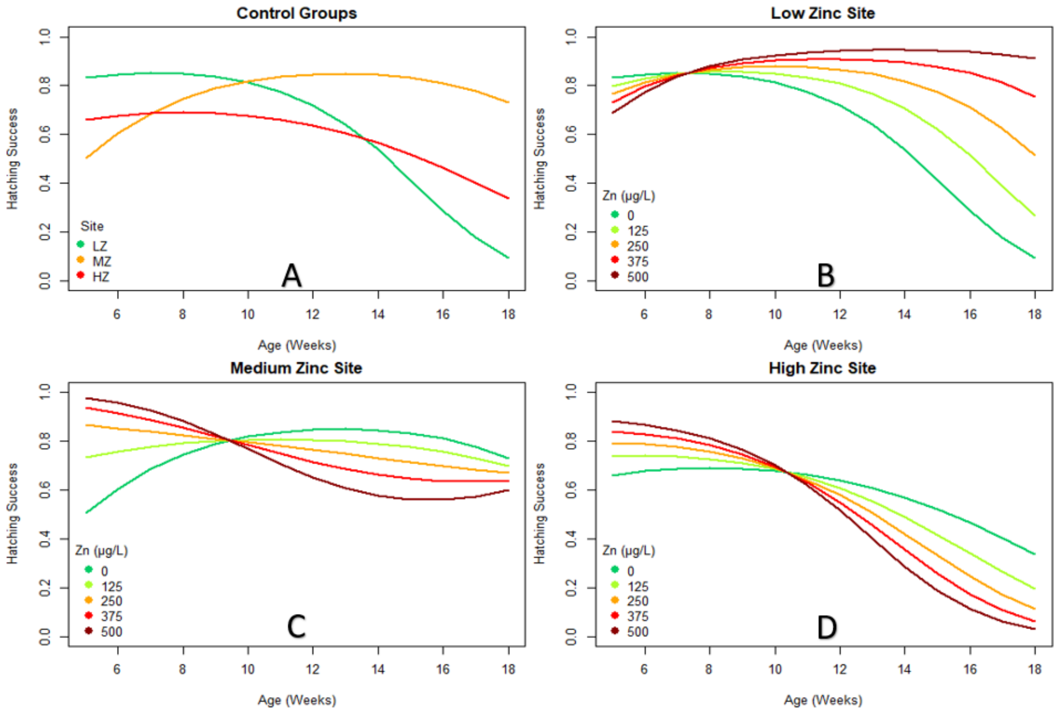

Figure 9. Predicted hatching success rates of offspring throughout a parental reproductive lifetime.

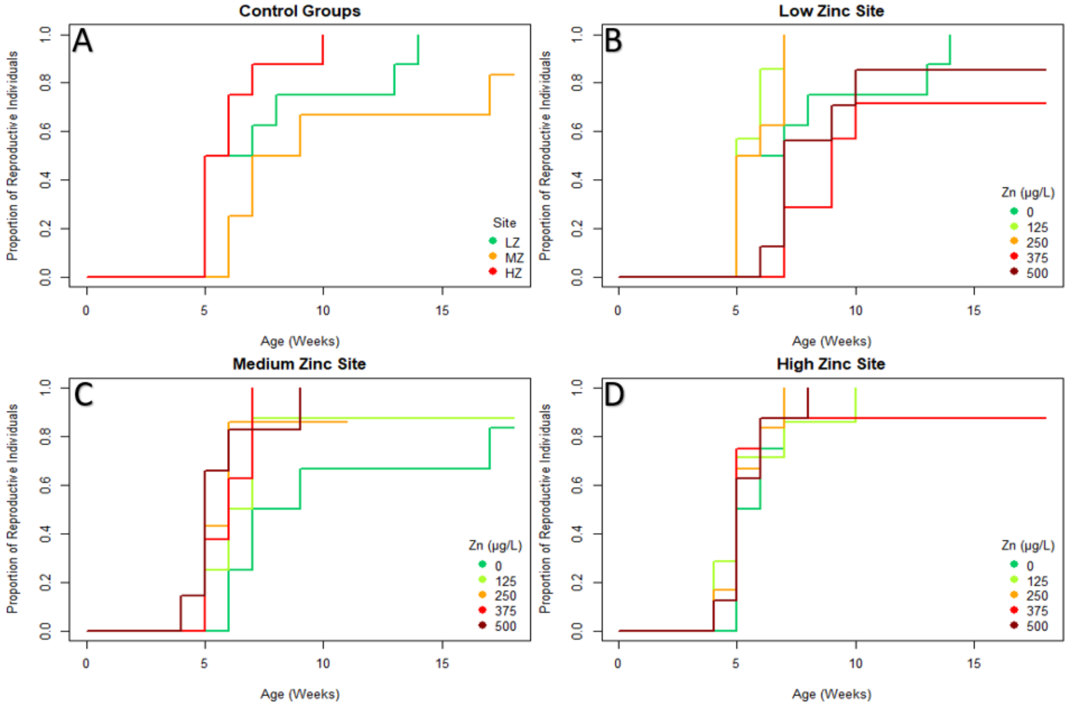

Figure 10. Proportion of reproductive F_1 individuals throughout the course of the study.

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