THE TIMING OF MATERNAL AND PATERNAL PREDATOR EXPOSURE AND RESULTING TRANSGENERATIONAL EFFECTS IN *PHYSA ACUTA*

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THE TIMING OF MATERNAL AND PATERNAL PREDATOR EXPOSURE AND RESULTING TRANSGENERATIONAL EFFECTS IN *PHYSA ACUTA*

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Abstract: The interactions that an individual has with its environment can impact the physical traits and behaviors of both that individual and its offspring. This phenotypic plasticity can influence the evolution of a population, or the relationships between species. Such is the case when organisms use cues from their environment to develop more effective defenses against predation. Currently, we do not have a consistent record of what factors are most influential in these effects or understand the mechanisms behind them, especially in hermaphroditic species. In this study, I investigated how the morphology and behavior of a hermaphroditic pond snail, *Physa acuta*, is affected by parental exposure to a predator cue, and how those effects differed based on the exposure of mothers versus fathers and the timing of those exposures. Two generations of snails were bred and raised in the laboratory. The parental F1 generation was exposed to one of three treatments: control cue, early exposure to a predator cue, or late exposure to a predator cue. The F2 generation was raised without exposure to a predator cue, then underwent two anti-predator behavior experiments once sexually mature. The behavioral trials assessed a snail's predator avoidance behavior when exposed to a predator cue, and the latency trials quantified if there was a difference in how quickly a snail exhibited this behavior, if at all, when exposed to a predator cue versus a control cue. I obtained soft body mass, shell mass, and shell morphometric data on both the F1 and F2 generations at sexual maturity. I found that the exposure of a parent to a predator cue affected some aspects of the phenotypes of their offspring. The timing of exposure had some effect on overall shell shape, but I found no apparent evidence for differences between maternal and paternal effects. My results indicate that exposure to a predator cue is the main factor contributing to both within-generation and transgenerational effects. Maternity or paternity and timing have less influential roles in plasticity, but effects may be revealed under more specific conditions such as when environmental information passed down from the parents is corroborated by the experiences of offspring.

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CHAPTER I

INTRODUCTION

Phenotypic plasticity refers to the ability of an organism to alter its phenotype based on the experiences it has in its environment and can occur both within and across generations. Transgenerational plasticity occurs when the life experiences and environments of an individual affect the phenotypes of that individual's offspring, including the expression of behavioral and morphological traits (Hellmann et al. 2020, Tariel et al. 2020a, Bell et al. 2019, Beaty et al. 2016). This can be viewed as a complex form of communication between a parent, the signaler, and its offspring, the receiver (Bell and Hellmann 2019). Different types of experiences (drought, conflict, competition) are known to affect different types of traits or alter the magnitude of the effects, and the presence or absence of certain hormones or nutrients can affect the specific resources parents are able to invest in offspring, thus altering a potentially wide variety of phenotypes (Gilad and Scharf 2019, Bonduriansky et al. 2016).The effects of environmental cues tend to be nonadditive, meaning that offspring who had an influential experience in their development (within-generation plasticity) and who also had a parent with an influential experience (transgenerational plasticity) do not experience effects more strongly, but experience the same effects at the same intensity as offspring who only experienced one type of plasticity (Stein et al. 2018). Exposure to cues does not necessarily need to be extensive either; short-term and long-term exposures can produce almost identical effects (Mikulski and Pijanowska 2010).

In the context of predator-prey interactions, transgenerational effects can lead to the development of traits like armor, weapons, or a change in size to avoid predation, among other defenses. Additionally, the parent may pass on information about their environment to their offspring to increase survival through behavioral mechanisms; one way this can present is by producing offspring that perform predator-avoidance behaviors more often or more dramatically when exposed to predator cues (Tariel et al. 2020a). Transgenerational plasticity can be adaptive in this manner because within-generation plasticity would first require an initial encounter with the stressor, which the individual may be ill-equipped for or not survive and would also require more time and resources to develop (Agrawal et al. 1999).

Transgenerational effects, however, are not always adaptive; they may have no effect on fitness or be maladaptive. Changes in the environment, inaccuracy of the information received by the parents, or the lack of resources to develop these traits may all contribute to a non-adaptive status (Hellmann et al. 2020). Because of this, the adaptive value of a trait is highly subject to change (Tariel et al. 2020b). It is also possible for multiple effects to arise from the same exposure, some being adaptive and some being maladaptive (Handelsman et al. 2013). A maladaptive trait may develop through changes in the body condition of an individual or their offspring, such as the effects of a drought or starvation (Tariel et al. 2020b).

In *Physa*, a genus of freshwater snails, a driving force in morphological and behavioral plasticity is the presence of predators. Several morphological changes may occur, including changes in body mass, size, the shape of the shell, and crush resistance. Within generations, predator exposure has been linked to the development of elongated shells and more narrow apertures (Beaty et al. 2016). Across generations, exposure of a parent to predator cue tends to cause the development of larger shells with greater crush resistance in offspring (Beaty et al. 2016). Behaviorally, parental exposure to a predator cue can affect the presence and intensity of anti-predator behaviors. A common predator of aquatic snails are crayfish, which hunt on the

floor of creeks and lakes, making it advantageous for snails to exit the water in their presence. The inclination to avoid predation conflicts with the snails' need to forage for algae on underwater surfaces. Transgenerational effects can influence how quickly a snail leaves the water when it senses danger, or if it leaves the water at all (Tariel et al. 2020a). However, these behavioral effects are not consistently observed in experiments (Beaty et al. 2016).

There is emerging evidence that transgenerational effects on offspring phenotype can differ depending on the experiences of mothers and fathers. For example, the effects on offspring may differ if a mother versus a father was exposed to a predator cue, if parental diets differed between the sexes, or if the parents each experienced different climactic stressors (Tariel et al. 2020a, Gilad and Scharf 2019, Bonduriansky et al. 2016). Some affected traits include behavior, physiology, life history, gene expression, environmental perception, and morphology (Hellmann et al. 2020, Tariel et al. 2020a, Tariel et al. 2020b). Stressors that affect body condition, such as starvation, can affect offspring production in would-be mothers, and alter the size of offspring when fathers are exposed (Gilad and Scharf 2019).

The exposure of one parent to a predator cue has a greater effect on offspring than when both parents are exposed, and perhaps parents may dilute or even cancel out each other's effects (Hellmann et al. 2020, Tariel et al. 2020a, Beaty et al. 2016). In most study systems for maternal or paternal transgenerational effects, the organisms involved are not hermaphroditic and exhibit some form of parental care. Males have more limited pathways than females to pass on information to their offspring, as mothers are known to influence their young through hormones, non-coding RNA, nutrients, and more. Additionally, maternal hormones and similar inducers are suspected to carry environmental information and persist in diluted forms across multiple generations (Yin et al. 2015, Agrawal et al. 1999). In some cases, paternal effects vary in the context of maternal effects, with paternal effects only being revealed when certain maternal experiences occurred, again emphasizing the impact of maternal experiences (Galloway 2001).

However, there is increasing evidence that paternal effects through epigenetic alterations may be more widespread than originally thought, and alteration of sperm phenotype based on environmental conditions is common and has been shown to affect offspring phenotype and fitness, especially when the environmental conditions of the offspring mirror the environmental conditions of the father (Crean and Bonduriansky 2014, Crean et al. 2013).

In hermaphroditic *Physa* snails, here is speculation that morphological traits, such as the size and crush resistance of shells across generations, may be more heavily influenced by maternal investment (Beaty et al. 2016). Maternal exposure to predator cue has also been linked to a decrease in predator avoidance behaviors, potentially an example of maladaptive transgenerational effects, although those effects have only been observed when the offspring themselves were also exposed to predator cue during development (Tariel et al. 2020a). Because individuals of a hermaphroditic species have similar ecology and gamete dispersal in the male and female roles, in contrast with individuals of a species with distinct males and females, differences in maternal and paternal transgenerational effects may be less pronounced (Tariel et al. 2020a).

Regardless of the sex of the parent, the experiences of each must be integrated effectively and accurately by the offspring in order for those offspring to optimally respond to their environments. When offspring receive different environmental cues from each parent there may be an issue of reliability and prioritization of information, especially in respect to the cues they themselves have received from their immediate environment (Tariel et al. 2020a). The reliability and accuracy of a cue from a parent will be dependent on how much time has passed between the perception of the cue by the parent and the expression of the phenotype by the offspring, especially in variable environments (Tariel et al. 2020b). The immediate environment tends to be the most reliable source of information, which is likely why the observability of transgenerational

behavior effects in *Physa* appears to be linked to the personal experiences of the offspring (Tariel et al. 2020a).

The timing of experiences is influential in the expression of both within-generation and transgenerational effects, but the critical windows and their significance have yet to be discovered in *Physa* snails. Furthermore, the identification of critical windows is inconsistent across other taxa in which it has been investigated (Tariel et al. 2020b). Expression of transgenerational effects has been studied in organisms after parental treatment early in life, parental treatment before reproduction, or parental treatment after reproduction, and effects have been documented in offspring at a variety of ages and after direct offspring (Tariel et al. 2020b). Two key life stages that have been identified as critical developmental windows across species are early development and at the time of reproduction, but there is still much debate and speculation surrounding the importance of these stages (Tariel et al. 2020b, Bell and Hellmann 2019, Donelson et al. 2018, McNamara et al. 2016, Fawcett and Frankenhuis 2015, Mikulski and Pijanowska 2010). Experiences shortly before or during reproduction are likely to have the most accurate information about the current environment, but in a stable environment, early experiences may be more influential due to the influence these experiences have on the behavior and morphology of the parents (Bell and Hellmann 2019, Donelson et al. 2018, McNamara et al. 2016). Reproductive maturity has also been known to correlate with the age of greatest sensitivity to environmental cues in some invertebrates (Mikulski and Pijanowska 2010). These critical windows may differ among taxa, which may be one explanation for the differences in transgenerational plasticity observed between studies. These life stages in which experiences are most likely to cause phenotypic effects are expected to play a vital role in both evolution and predator-prey interactions (Tariel et al. 2020b).

On the parental side, timing may be important due to the ability of the nervous system to receive and process environmental cues in each developmental stage, as an egg would not be able

to receive a cue the same way that a mature organism would. Receiving a cue after the development of a trait is too far along to undergo alterations would not be beneficial, the same way that exposure to a cue before the organism can interpret its meaning would not be beneficial (Bell and Hellmann 2019). Because of differences in the ability to receive cues across developmental stages, timing can affect the mechanisms of informational transfer and therefore the changes seen in the next generation (Donelson et al. 2018). Considering this information through the lens of adaptability, critical windows are most likely to be found in life stages when environmental conditions are uncertain, there are many cues to receive and process, and an individual is able to alter its phenotype with relative ease (Fawcett and Frankenhuis 2015).

In this study I tested for the presence of maternal and paternal transgenerational effects in a pond snail, *Physa acuta*, and compared the magnitudes of these effects and tested how they are each affected by the timing of parental exposure to predator cues. Because these snails do not provide paternal care and are kept in individual containers throughout their lifespan in this experiment, the only parental effects from either parent are pre-fertilization. This setup allows me to be certain that any effects on the offspring are due to either parental experience or to physical factors such as genetics or nutrients. The two main questions I addressed with this experiment were:

1) Does a mother's or father's exposure to predator cues have a greater effect on the phenotypes of their offspring?

2) How does the timing of exposure to predator cues affect maternal and paternal transgenerational effects? Do stressful experiences earlier in a snail's life or closer to the time of reproduction have a larger effect on offspring phenotypes and do timing effects differ between maternal and paternal effects? This will help identify critical windows for parental experiences to

affect offspring through transgenerational plasticity and determine if one of these exposure periods causes more lasting effects than the other.

I expected that maternal effects would be larger than paternal effects, because eggs carry more resources than sperm and thus, the mother's experiences may have greater influence than the father's experiences. Additionally, these differences may be emphasized if the parents receive conflicting cues about the safety of their environment. For example, if the mother experienced a safe environment while the father experienced one with predators, anti-predator behavior may be less pronounced in the offspring than it would be if the mother were exposed to predators. The critical windows for transgenerational effects- stages in development where experiences are more likely to influence offspring- may coincide with the development of eggs and sperm. Early experiences may influence individuals, or parents, throughout their lives, but more recent experiences may have stronger effects on offspring. I expected to observe a difference in the intensity of the behavioral and morphological effects on the offspring based on which parent was exposed to a potentially dangerous environment, with the mother's experiences having greater influence.

CHAPTER II

METHODOLOGY

Fifty wild *Physa acuta* snails were identified and collected from Sanborn Lake in Stillwater, Oklahoma (UTM: 36°15′36.6″N, 97°07′61.5″W) on May 18, 2021, and again on May 25, 2021. All treatments, measurements, and behavioral trials were done in two blocks to ensure consistency in the age of the snails at the time of each manipulation. After collection, I transported the snails to the lab in a plastic tub filled with lake water. I refer to these snails as the F0 generation. I divided the F0 snails into groups of ten, housed them in five 1.7 L Pyrex containers with 1 L dechlorinated water, and allowed them to breed and lay eggs for approximately 48 hours. I removed the egg masses for the F1 generation, from the F0 tank, and placed them into individual 16 oz deli cups with 300 mL dechlorinated water for 10 days awaiting hatching. I randomly selected 140 hatched snails and reared them individually in deli cups for five weeks. Twice a week I gave these snails fresh water and fed them, 10 mg of algae through week three and 15 mg of algae after week three.

Crayfish (*Procambarus sp.*) originally obtained from Sanborn Lake were housed in the lab, with each crayfish occupying a 1.7 L tank with 600 mL of dechlorinated water. Predator cue was made by combining a solution of 400 mL dechlorinated water and 3 g of crushed *P. acuta* with 80 mL of water from a crayfish tank one hour after the crayfish consumed 0.3 g of live *P. acuta* prey. This cue combination has been the most effective at eliciting predator avoidance

behavior from snails in a laboratory setting (Alexander and Covich 1991). I strained predator cue and froze it in 2 mL aliquots, which were thawed in batches throughout the study and used in 1 mL doses. Twenty-eight F1 snails were given a dose of crayfish predator cue one week after hatching and received a second and final dose later that week. These are the "early predator experience" snails. Twenty-eight F1 snails were given a dose of crayfish predator cue four weeks after hatching and received a second and final dose later that week. These are the "late predator experience" snails. 84 F1 snails were given 1 mL doses of control cue (thawed aliquots of dechlorinated water) during the early exposure and late exposure periods.

The F1 snails were paired for mating. At five weeks old, I marked snails with nail polish on their shell for identification. I paired them with one other snail in a deli cup and left them for 48 hours. Afterwards I moved each snail back to its own individual deli cup. As the snails are hermaphroditic, each one in the pair acts as both the mother and the father. When a snail lays eggs in its own deli cup, it is the biological mother of those offspring while the other snail they were in the breeding cup with is very likely to be the father of those offspring (but a low rate of selfing is possible). Therefore, the 70 pairs resulted in 140 combinations of maternity and paternity and predator cue exposure since each snail played both biological parental roles. There were 28 combinations of F1 snails where the mother was not exposed to predator cue and the father had the early predator experience. There were 28 combinations of F1 snails where the mother was not exposed to predator cue and the father had the late predator experience. There were 28 combinations of F1 snails where the mother had the early predator experience and the father was not exposed to predator cue. There were 28 combinations of F1 snails where the mother had the late predator experience and the father was not exposed to predator cue. Finally, there were 28 combinations of F1 snails where neither the mother nor the father was exposed to predator cue (Figure 1).

Pairs were allowed to mate for 24 hours, and then I moved them to individual deli cups. After another 48 hours, I collected the egg masses laid in those individual cups and recorded the size and number of egg masses. The size of each individual egg mass was scored as tiny or normal. I developed a points system to quantify the size of each egg mass, with tiny egg masses being worth 0.5 "points" and normal egg masses being worth 1 "point". I then took the sum of points for all the individual egg masses in a cup (for example, if one tiny and two normal egg masses were present, the overall score used in data analysis would be 2.5). From each individual cup which was occupied by one F1 mother, I randomly selected five hatched snails and moved them all into a single cup. I kept an additional backup cup of five F2 snails from each mother so that any casualties in the first cup could be replaced, and therefore the population density would remain the same. These F2 snails were not exposed to predator cues. They received fresh water and food twice a week. Until they were three weeks old, each group of maternal siblings was given 50 mg of algae; after three weeks old, they were given 75 mg of algae.

After the F1 snails laid eggs, I extracted the soft body from the shell to obtain the mass of each snail and photographed the shell under a dissecting microscope for measurement and shape analysis. At five weeks old, the F2 snails were also extracted, weighed, and photographed.

I also conducted behavioral trials with the F2 snails. I randomly selected two F2 snails (the first two encountered when searching the cup in a clockwise motion) from each cup of five and fasted them for 24 hours. I then placed each of these F2 snails into a deli cup, with food and 300 mL dechlorinated water. The snail's position in the cup (a binary measurement of above (1) or below (0) the water line) was observed and recorded every fifteen minutes for one hour. After this one hour, snails were below the water and I administered a 1 mL dose of predator cue into the cup and recorded the binary position of the snails every fifteen minutes for one hour.

Four days after the behavioral trials, I conducted latency trials. One of the three remaining F2 snails in each cup was randomly selected to participate in these trials. I placed snails into a cup filled with dechlorinated water to the 2.8 cm mark; the snails were constrained to the bottom of the cup in a weighted, bottomless mesh cage. I administered a 1 mL dose of control cue, and 60 seconds later released the snails from their mesh containers at the bottom of the cup. The snails had up to five minutes to exit the water, and the time it took for each snail to do so was recorded. Exiting the water was defined as a portion of the snail's soft body mass breaking above the water line. After the control trials, the same steps were repeated with predator cue.

From the photographs of the F1 and F2 snails I measured shell length, shell width, aperture length, aperture width, and spire length using the ImageJ software, following the relevant methods outlined by DeWitt et al. (2000). The ratio of shell length and width illustrates how globular or oblong the overall shell shape is, and consequently the general ability of that shell to resist being crushed by a predator. Aperture length and width would affect the ability of predators to extract a snail's soft body mass from its shell. A longer spire length would allow the snail to hide from a predator deeper in its shell.

F1 data were analyzed using a generalized linear mixed model (GLMM) with F0 egg mass ID as a random effect. The production of viable offspring was the only dataset analyzed with dyad ID (the unique ID number given to a mated pair of snails, regardless of who was the biological mother or father) as a random effect (as opposed to F0 egg mass ID). A Poisson distribution was used in the analysis of number of egg masses laid. Models were ranked by the amount of support they received from the data using Akaike Information Criterion (AIC). The model with the smallest ΔAIC is the one receiving the most support from the data. For the F1 snails the alternative models were the null model, the exposure model (exposed or not exposed), and the timing model (none, early, or late).

F2 data, including behavioral and latency trials, were analyzed using a GLMM with F2 cup ID as a random effect to keep track of the effects on maternal siblings. For the F2 snails the alternative models were the null model, the exposure model, the timing model, the parent model (none, maternal, or paternal), and the parent and timing model (none, maternal early, maternal late, paternal early, or paternal late). These models were also ranked using AIC.

A beta distribution was used for the proportional data, such as shell mass as a proportion of total mass. The distribution of total shell mass in F2 snails was not normal but taking the log of those measurements fixed the distribution issues. There were convergence issues with the proportion of spire length to shell length F2 data as well, which was remedied by centering the data. Latency trial data were converted to a binary "exited the water" or "did not exit the water". The difference between control cue escape times and predator cue escape times in the latency trials was analyzed as well using the same GLMM and models as the original latency data.

For the analysis of behavioral trials, all models consisted of the cue administered in the trial (control versus predator) and the addition of (+) or interaction with (*) the effects from parental exposure, the timing of parental exposure, maternal or paternal effects, and the combination of maternity and paternity and exposure timing. There was also a null model and a model for solely the administered cue. These were the only models that included additive and interactive effects.

A total of 229 F1 snails and 632 F2 snails were used in data collection. I obtained mass data from 229 F1 snails and 522 F2 snails, and morphometric data for the shells of 196 F1 snails and 401 F2 snails. Extraction complications and unclear photographs resulted in some of the snails being unusable for mass and morphometric data collection. Of the F1 snails, 221 produced egg masses. Of the F2 snails, 406 were used in behavioral trials and 226 in latency trials. I obtained usable behavioral data from 400 of those snails and usable latency data from 192 of the

snails. Untimely escapes due to inability to contain the snails were the main reasons for unusable data in behavioral and latency trials.

CHAPTER III

RESULTS

Effects on the morphology of F1 snails

I found evidence that predator treatment and the timing of the predator treatment affected some of the measured phenotypes of the parental snails (Table 22). Exposure to predator cues affected shell length relative to shell width (shell length / shell width) (Table 1) with individuals exposed to predator cues having relatively shorter shells (1.79 ± 0.01 SE) than control snails (1.82 ± 0.01 SE) (Figure 2). The timing of exposure to predator cues appears to have affected the proportion of the snail's total mass that was composed of their shell's mass (shell mass / total mass) (Table 2), hereafter referred to as proportion shell mass, with the proportion of a snail's total mass that was their shell mass being higher when they were exposed to predator cue later in life (0.35 ± 0.01 SE) than when not exposed (0.33 ± 0.01 SE) or exposed earlier in life (0.33 ± 0.01 SE) (Figure 3). The timing of exposure to predator cues also appears to have affected aperture length relative to shell length (aperture length / shell length) (Table 3) with apertures being relatively longer when individuals were exposed to predator cue earlier in life (0.59 ± 0.01 SE) than when not exposed (0.57 ± 0.01 SE) or exposed later in life (0.57 ± 0.01 SE) (Figure 4). Total mass (Table 4), shell length (Table 5), spire length / shell length (Table 6), and aperture length (Table 7) were all best explained by the null model and thus do not appear to have been affected by exposure or the timing of exposure to predator cues.

Effects on the reproduction of F1 snails

The number of egg masses laid by F1 snails was affected by exposure to the predator cue (Table 8) with F1 snails laying more egg masses when exposed to predator cue (2.16 ± 0.17 SE) than did control snails (1.82 ± 0.13 SE) (Figure 5). Exposure to a predator cue also affected the size of egg masses (Table 9) with exposure to a predator causing a greater sum of sizes (2.02 ± 0.15 SE) than exposure to control cue only (1.84 ± 0.14 SE). Whether pairs of F1 snails successfully produced an F2 offspring was unaffected by predator cue exposure, which parent was exposed, or the timing of the exposure (Table 10).

Effects on the morphology of F2 snails

I found evidence that the predator treatment and the timing of the predator treatment affected some of the measured phenotypes of the F2 snails. The exposure of a parent snail to predator cue affected the total mass of F2 snails (Table 11). Snails whose parents were not exposed to predator cue had a greater total mass (12.6 mg ± 1.6 SE) than snails who had a parent exposed to predator cue (10.1 mg ± 0.7 SE) (Figure 6). The proportion of aperture length to shell length was also affected by exposure of a parent (Table 12) with snails whose parents were exposed to predators having longer apertures relative to shell length (0.61 ± 0.01 SE) than snails that did not have parents exposed to predator cues (0.60 ± 0.01SE), but the difference was rather small (Figure 7). There was also some evidence that the timing of exposure and which parent was exposed explained the proportion of aperture length to shell length better than the null, however the extra complexity of those models was not favored over the simpler model that only had whether a parent was exposed or not. The timing of parental exposure to predator cue affected the

proportion of mass that was shell mass (Table 13). Snails whose parents were exposed to predator cues early had a greater proportion that was shell mass (0.45 ± 0.01 SE) than the offspring that had parents exposed later (0.43 ± 0.01 SE) or parents that were not exposed to predator cues (0.43 ± 0.01 SE) (Figure 8). Shell length (Table 14), shell length / shell width (Table 15), spire length / shell length (Table 16), and aperture length (Table 17) measurements were best explained by the null model, with no apparent effects due to parental exposure to predator cues or the timing of that exposure.

Effects on the behavior of F2 snails

In the behavioral trials, the snails responded to the presence of the predator cue. The location of the snails in the cups was best explained by whether the predator cue was administered or not (Table 18). Control cue was less likely to elicit an antipredator response in the snails (0.01 ± 0.01 SE) than predator cue (0.09 ± 0.02 SE). The second-best model was the interaction of administration of the cue with whether an F1 parent was exposed to predator cue or not (independent of maternity, paternity, or timing) (Table 18). The control cue was less likely to elicit an antipredator response, but parental exposure to predator cues may have increased responses to predator cues in the F2 snails (control cue, no parental exposure (0.01 ± 0.01 SE); control cue, parental exposure (0.01 ± 0.01 SE); predator cue, no parental exposure (0.07 ± 0.02 SE); predator cue, parental exposure (0.10 ± 0.02 SE)). This model was almost the best supported model despite having two more explanatory variables. The interaction of control and predator cue administration to the F2 snails and the timing of parental F1 snail exposure (independent of maternity and paternity) was the next best model (Table 17). The null model was the worst fit (Table 18).

In the latency trials, the exposure of an F1 parent snail to predator cue (independent of maternity and paternity) was the best explanatory model for whether the F2 snails left the water or not when exposed to control cues (Table 19) and to predator cues (Table 20). When the parents were not exposed to predator cue, the snails were more likely to leave the water when exposed to control cue (0.76 ± 0.07 SE) versus snails whose parents were exposed to predator cue (0.58 ± 0.04 SE). In response to predator cue, snails were more likely to exit the water if the parents had not been exposed to predator cue (0.77 ± 0.07 SE) than if the parents had been exposed to predator cue (0.59 ± 0.04 SE). The differences in latency between control cue administration and predator cue administration to F2 snails during these trials were not explained by any aspects of treatment of F1 snails (Table 21).

CHAPTER IV

DISCUSSION

I conducted this experiment to investigate how the exposure of a mother or of a father snail to a predator cue would affect the behavioral or morphological phenotypes of their offspring and whether those effects differ due to the timing of that parental exposure. I hypothesized that maternal effects would be larger than paternal effects. I also expected to find differences between the snails who had a parent who was exposed to predator cue early in life and the snails who had a parent who was exposed to predator cue later in life closer in time to when they reproduced. However, I found no evidence for differences between maternal and paternal effects, and little evidence for differences due to the timing of exposure to predator cues.

My results suggest that, when offspring are raised in a controlled environment without their own exposure to predator cues, the effects of within-generation plasticity and transgenerational plasticity are similar for both F1 parent snails and F2 snails, with exposure to a predator cue (not dependent on maternity/paternity or timing) or parental exposure to a predator cue being the most influential treatment (Table 22). The morphological responses to predator cues that I observed were a combination of adaptive and maladaptive responses.

The predator avoidance behavior of offspring who had a parent who was exposed to a predator cue is arguably maladaptive compared to that of offspring whose parents were not exposed to a predator cue. I believe the higher population density of F2 snails and the lack of exposure to predators before they reached sexual maturity were likely the two largest factors in the low number of adaptive responses I observed in the F2 snails (Tariel et al. 2020a).

Morphology

Exposure to a predator cue affected phenotypes both within and across generations. In the F1 snails who underwent these treatments, timing affected the proportion of aperture length to shell length and the proportion of shell mass to total mass, although the effects were most pronounced in individuals exposed early and late, respectively. The only transgenerational effect best explained by the timing of predator cues was the proportion of shell mass to total mass. F2 snails who had a parent who was exposed to a predator cue early in life had a greater relative shell mass than those who were exposed late or not at all.

The effects of treatments on the phenotype of F1 and F2 snails, and the traits that were affected versus the ones that were not affected, were often similar (Table 22). Shell length, spire length / shell length, and aperture length were all best explained by the null model for both F1 and F2 snails, and thus they were unaffected by exposure of the F1 snails to predator cues. The proportion of a snail's mass that was their shell mass was larger in both F1 and F2 snails when an F1 parent snail was exposed to predator cue, although there were some differences due to the timing of that exposure. The proportion of aperture length to shell length experienced similar effects. I found evidence of potentially adaptive within-generation and transgenerational plasticity in morphological traits when an F1 parent snail experienced a predator cue. The proportion of a snail's mass that was their shell mass was most strongly affected by the timing of the cues, however the effects differed between F1 and F2 snails.

In F1 snails, late exposure to the predator cue caused a greater relative shell mass, whereas in F2 snails, early exposure of a parent to a predator cue caused a greater relative shell mass. In the F1 snails, this may be explained by the snails growing thicker shells when exposed to predator cue. The timing would be vital in this case because when snails are younger and smaller, the added shell mass would be more minimal, and the additional growth may not continue past the exposure period. The older and larger snails would have a larger shell mass already, and the same bulking to that shell would create a larger shell mass change than in the younger snails. Bulking later in life might also cost more resources and energy (Agrawal et al. 1999). The larger shell mass proportion of the F2 snails may be indicative of a critical window in which the parents' experiences affect their offspring's phenotypes.

Measurements of proportions, such as shell mass / total mass and aperture length / total length, were most affected by the treatment, suggesting that the overall shape of the shell is affected by predator exposure more than single measurements such as length or width. The shape of the shell affects the ability of a snail to hide in the shell, avoid physical extraction, and resist crushing attempts (DeWitt et al. 2000). One explanation for the observation of proportion or shape differences and not individual length or width differences is that the small deli cups and the population density in F2 cups may have not allowed for size differences, since the density and the lack of F2 predator exposure were the only differences in husbandry between generations, but population density would not explain the effects seen in the F1 generation. Since there was an abundance of food, lack of energy is not a likely explanation. The only proportion measurement that was not affected by treatment was spire length / shell length. A longer spire is expected to allow a snail to hide deeper in its shell and avoid extraction by predators. The lack of an effect on the proportion of spire length to shell length is unexpected, considering that crayfish extract snails and longer spires would have provided the snails with an advantage in this environment (DeWitt et al. 2000).

Exposure to a predator resulted in a greater number of egg masses produced by F1 snails and led to a greater sum of sizes. There were no observed effects on whether a snail produced successful offspring or not. In an environment where there is a known risk of predation, producing a greater number of egg masses may ensure that at least one of those masses survives. Exposure to a predator cue might also lead snails to produce more egg masses early in life at the cost of fewer egg masses later in life (Abbey-Lee and Dingemanse 2019). The higher sum of sizes suggests that snails exposed to predators are not disadvantaged in reproductive output, even receiving some benefit from their exposure, and that egg mass size is not substantially reduced either. Exposure to a stressful environment may encourage the snails to increase their reproductive output and efforts, so that even if they do not survive long themselves, they are still likely to produce successful offspring; this would support the terminal investment hypothesis, which has been investigated in other species (de Moraes et al. 2019, Brannelly et al. 2016, Krams et al. 2015).

Likewise, I found evidence of maladaptive within-generation and transgenerational plasticity in morphological traits when an F1 parent snail experienced a predator cue. The proportion of aperture length to shell length in F1 snails was affected by the timing of exposure to predator cues and in F2 snails by whether parents were exposed to predator cues. In F1 snails, early exposure to predator cues caused relatively longer apertures. In F2 snails, exposure to predator cues at any time also caused relatively longer apertures. Snails may take refuge in their shells when they are unable to flee from a dangerous environment, and larger apertures allow for predators to access the interior of the shell and extract a snail more easily (DeWitt et al. 2000). The proportion of shell length to shell width was affected by exposure in F1 snails, with exposure to a predator resulting in relatively shorter shells, but the same response variable was best explained by the null model in F2 snails. Shortening the length of the shell relative to its width produces a more globular-shaped shell. There are mixed accounts of whether shell shape affects

crush resistance, but in cases where it was found to be a significant factor, globular shells were more beneficial in habitats with crushing predators such as fish, and elongated shells were more beneficial in habitats with extracting predators such as crayfish (Beaty et al. 2016, DeWitt et al. 2000).

Behavior

In the behavior and the latency trials, the exposure of a parent to predator cue at any time made the F2 snails less likely to respond to both control cue and predator cue. The administration of control cue versus predator cue was the most supported model in the behavioral trials. Differences in latency times between snails of control parents and of predator-exposed parents could not be explained by treatment. These data may suggest that parental exposure to predators could be useful in reducing the number of false positives detected by offspring regarding the perception of danger in their environment and allow for more foraging time and less energy expended attempting to avoid predators, particularly when the offspring are smaller and less at risk of predation (Catano et al. 2016, Ings and Chittka 2008). However, the trade-off appears to be that fewer individuals flee when faced with predation risk. One explanation is that the individuals choose not to respond to these cues, as they may be less fearful of potential threats in their environment. The smaller size of the parent-exposed F2 snails supports this, as they are potentially less likely to be a target of predation and have fewer resources and reproductive potential to defend (Catano et al. 2016).

We must consider, of course, the differences in phenotypes between these individuals as well. Lower total mass and greater relative shell mass (when a parent is exposed early) may serve to lessen the predation risk by making the individual a less appealing or less detectable target with a larger and bulkier armored structure to hide in. If these are effective adaptations to avoid

predation, it may be more beneficial to stay put and continue foraging than to attempt to escape, especially since the smaller individuals must expend more energy to cover distance than larger individuals (Catano et al. 2016). It is also interesting to note that, in the latency trials, the likelihood of an F2 snail leaving the water was almost identical, regardless of control cue versus predator cue administration, between snails whose F1 parents had the same cue exposures. This lack of response to predator cue may be because the offspring were all raised without predator cue exposure, and therefore they did not have the necessary early experiences needed to integrate the environmental information received from their parents into their own perception of their environment (Tariel et al. 2020a).

Conclusions

Some of my results and the experimental design suggest that elevated population density could affect within-generation plasticity and potentially mask transgenerational effects (Tariel et al. 2020b). Total mass was not affected by treatment in F1 snails, but in F2 snails, parental exposure resulted in offspring with lower total mass. One possible explanation for this is that exposure to a predator cue reduced foraging by the snails, and although this was not enough to affect the total mass of F1 snails, the parents may have had fewer nutrients and resources to allocate to eggs or sperm, or to spermatophores (Koene 2017). Another potential explanation is that the stress and resulting hormones affected the gametes (Yin et al. 2015, Agrawal et al. 1999). The F2 snails were smaller in size and total mass than the F1 snails, despite having been raised in almost identical laboratory conditions, obtaining the same amount of food and being the same age at the time of data collection. The one difference is that F2 snails were housed in groups of 5, whereas F1 snails were housed individually. Despite being given five times the food, F2 snails were not given five times the space, and the population density may have affected growth

(Cannarsa and Meconcelli 2017). Competition for food is not likely, as leftover food was common at each water change and feeding, but competition for space or for other non-food-based nutrients may have played a role.

Future research could investigate how population density affects transgenerational effects, since the only difference between husbandry of my larger F1 snails and smaller F2 snails was an increased density in the F2 cups. Although competition for food was not likely a factor, there may have been other minerals or nutrients in the water or air that were limiting and affected phenotypic expression or lack of space itself could have been a constraint. A better understanding of when in a snail's life cycle each gamete is produced is essential in identifying any potential critical windows. If eggs or sperm are already developed at the time of a predator exposure, there is less opportunity to adjust phenotypes and share environmental information with offspring. Likewise, if too much time has passed between gamete production and predator exposure, the cues may not be reliable enough to pass on or implement (Bell and Hellmann 2019). Maternal and paternal effects may be less likely in this study system, especially without offspring predator exposure prior to sexual maturity (Tariel et al. 2020a).

Identifying the variables that have the greatest influence on transgenerational effects will lead to a greater understanding of transgenerational plasticity, and to what extent anti-predator behaviors depend on parental experience and the timing of that experience. This will provide additional insight into how organisms adapt to their environments and pass on traits to their progeny through mechanisms other than genetic inheritance.

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Model	ΔAIC	df	w
Exposure	0	4	0.41
Timing	0.3	5	0.35
Null	1.0	3	0.24

Table 1

Model comparison based upon Akaike Information Criterion for shell length / shell width of F1 snails.

Model	ΔAIC	df	w
Timing	0	5	0.83
Exposure	4.1	4	0.11
Null	5.0	3	0.07

Table 2

Model comparison based upon Akaike Information Criterion for shell mass / total mass of F1 snails

Model	ΔAIC	df	w
Timing	0	5	0.75
Exposure	3.0	4	0.17
Null	4.3	3	0.09

Table 3

Model comparison based upon Akaike Information Criterion for aperture length / shell length of F1 snails.

Model	ΔAIC	df	w
Null	0	3	0.64
Exposure	1.8	4	0.26
Timing	3.8	5	0.10

Table 4

Model comparison based upon Akaike Information Criterion for total mass of F1 snails.

Model	ΔAIC	df	w
Null	0	3	0.56
Timing	1.8	5	0.23
Exposure	1.9	4	0.21

Table 5

Model comparison based upon Akaike Information Criterion for shell length of F1 snails.

Model	ΔAIC	df	w
Null	0	3	0.55
Exposure	1.1	4	0.32
Timing	2.9	5	0.13

Table 6

Model comparison based upon Akaike Information Criterion for spire length / shell length of F1 snails.

Model	ΔAIC	df	w
Null	0	3	0.56
Exposure	1.1	4	0.32
Timing	3.1	5	0.12

Table 7

Model comparison based upon Akaike Information Criterion for aperture length of F1 snails.

Model	ΔAIC	df	w
Exposure	0	3	0.51
Null	1.1	2	0.30
Timing	1.9	4	0.20

Table 8

Model comparison based upon Akaike Information Criterion for number of egg masses laid by F1 snails.

Model	ΔAIC	df	w
Exposure	0	4	0.45
Null	0.4	3	0.38
Timing	1.9	5	0.18

Table 9

Model comparison based upon Akaike Information Criterion for egg mass size in F1 snails.

Model	ΔAIC	df	w
Null	0	2	0.55
Exposure	2.0	3	0.20
Timing	2.7	4	0.14
Parent	3.9	4	0.08
Parent and Timing	5.3	6	0.04

Table 10

Model comparison based upon Akaike Information Criterion for the production of successful offspring by F1 snails.

Model	ΔAIC	df	w
Exposure	0	4	0.36
Null	0.5	3	0.29
Timing	1.4	5	0.18
Parent	1.8	5	0.14
Parent and Timing	5.1	7	0.03

Table 11

Model comparison based upon Akaike Information Criterion for total mass of F2 snails.

Model	ΔAIC	df	w
Exposure	0	4	0.39
Timing	0.8	5	0.26
Parent	2.0	5	0.14
Null	2.0	3	0.14
Parent and Timing	3.7	7	0.06

Table 12

Model comparison based upon Akaike Information Criterion for aperture length / shell length of F2 snails.

Model	ΔAIC	df	w
Timing	0	5	0.33
Null	0.5	3	0.26
Exposure	0.7	4	0.23
Parent and Timing	2.3	7	0.10
Parent	2.7	5	0.09

Table 13

Model comparison based upon Akaike Information Criterion for shell mass / total mass of F2 snails.

Model	ΔAIC	df	w
Null	0	3	0.36
Exposure	0.1	4	0.33
Timing	1.9	5	0.14
Parent	2.1	5	0.13
Parent and Timing	4.1	7	0.05

Table 14

Model comparison based upon Akaike Information Criterion for shell length of F2 snails.

Model	ΔAIC	df	w
Null	0	3	0.39
Exposure	0.2	4	0.34
Parent	2.2	5	0.13
Timing	2.2	5	0.13
Parent and Timing	6.0	7	0.02

Table 15

Model comparison based upon Akaike Information Criterion for shell length / shell width of F2 snails.

Model	ΔAIC	df	w
Null	0	3	0.41
Timing	1.9	5	0.16
Parent	1.9	5	0.16
Exposure	2.0	4	0.16
Parent and Timing	2.6	7	0.11

Table 16

Model comparison based upon Akaike Information Criterion for spire length / shell length of F2 snails.

Model	ΔAIC	df	w
Null	0	3	0.46
Exposure	1.0	4	0.28
Timing	2.9	5	0.11
Parent	2.9	5	0.11
Parent and Timing	4.7	7	0.05

Table 17

Model comparison based upon Akaike Information Criterion for aperture length of F2 snails.

Model	ΔAIC	df	w
Cue Administered	0	3	0.25
Cue Administered * Exposure	0.3	5	0.22
Cue Administered * Timing	0.6	7	0.18
Cue Administered + Exposure	1.6	4	0.11
Cue Administered + Parent and Timing	2.5	11	0.07
Cue Administered * Parent	2.9	7	0.06
Cue Administered + Parent	3.1	5	0.05
Cue Administered + Timing	3.6	5	0.04
Cue Administered + Parent and Timing	6.5	7	0.01
Null	232.2	2	<0.001

Table 18

Model comparison based upon Akaike Information Criterion for behavior of F2 snails.

Model	ΔAIC	df	w
Exposure	0	3	0.42
Timing	1.0	4	0.26
Parent	2.0	4	0.16
Null	2.9	2	0.10
Parent and Timing	3.5	6	0.07

Table 19

Model comparison based upon Akaike Information Criterion for latency of F2 snails when exposed to control cue.

Model	ΔAIC	df	w
Exposure	0	3	0.41
Timing	0.8	4	0.27
Parent	2.0	4	0.15
Parent and Timing	3.1	6	0.09
Null	3.3	2	0.09

Table 20

Model comparison based upon Akaike Information Criterion for latency of F2 snails when exposed to predator cue.

Model	ΔAIC	df	w
Null	0	3	0.48
Exposure	1.2	4	0.26
Timing	2.9	5	0.11
Parent	3.2	5	0.10
Parent and Timing	4.8	7	0.04

Table 21

Model comparison based upon Akaike Information Criterion for difference in latency of F2 snails between predator and control trials.

Variable	F1	F2
Number of Egg Masses Laid	Exposure: P more egg masses laid	
Production of Successful Offspring	Null	
Egg Mass Score	Exposure: P greater egg mass score	
Total Mass	Null	Exposure: P lower total mass
Shell Mass / Total Mass	Timing: P late greater relative shell mass	Timing: P early greater relative shell mass
Shell Length	Null	Null
Shell Length / Shell Width	Exposure: P relatively shorter shells	Null
Spire Length / Shell Length	Null	Null
Aperture Length	Null	Null
Aperture Length / Shell Length	Timing: P relatively longer apertures when exposed early	Exposure: P relatively longer apertures
Control Cue Response: Behavior		Exposure: P less likely to respond to control cue
Predator Cue Response: Behavior		Exposure: P less likely to respond to predator cue
Control Cue Response: Latency		Exposure: P less likely to respond to control cue
Predator Cue Response: Latency		Exposure: P less likely to respond to predator cue
Difference in Latency Responses		Null

Table 22

Comparison of trait effects between F1 and F2 snails.

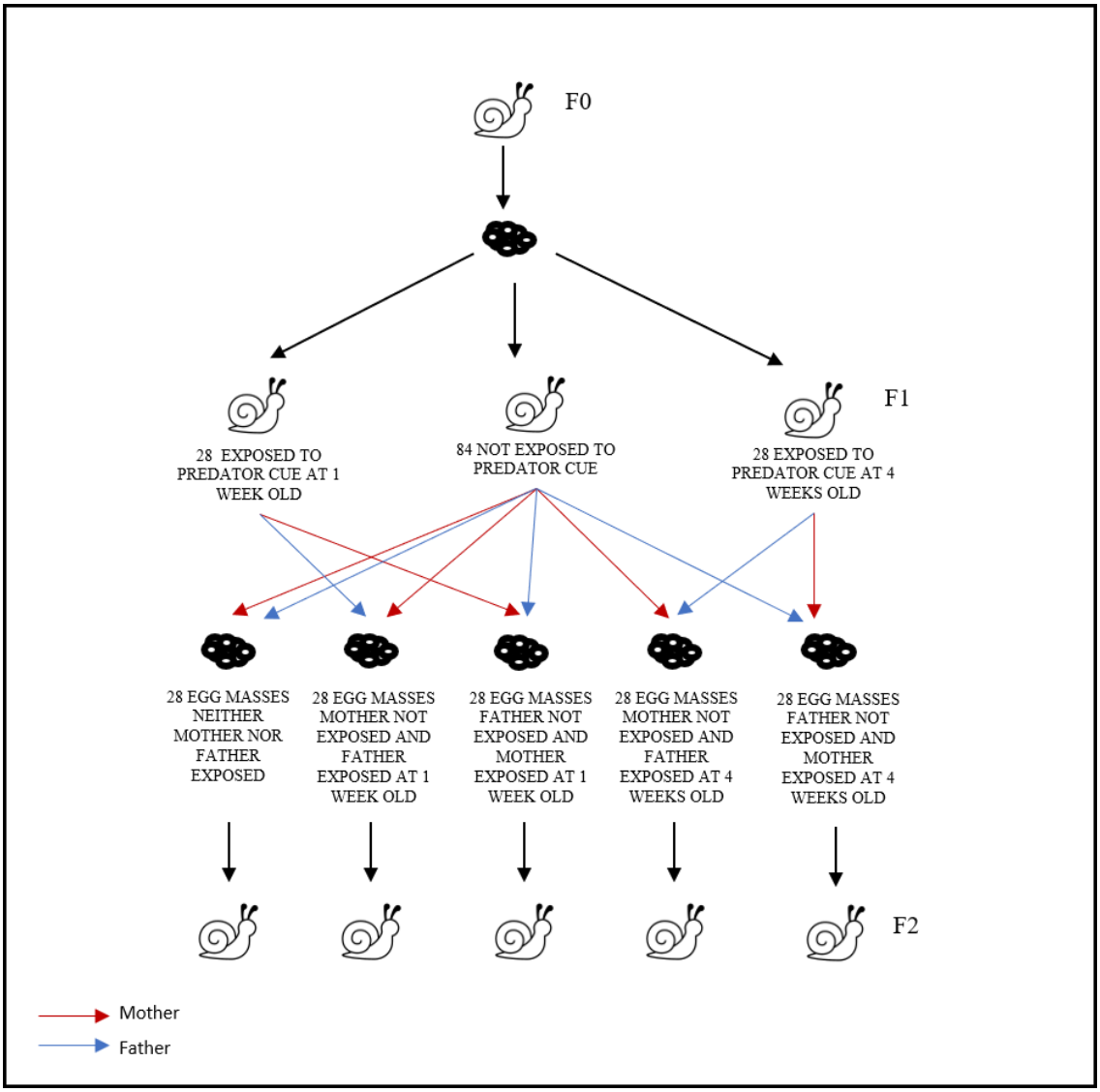

Figure 1

Diagram of snail cue exposure and breeding pairs.

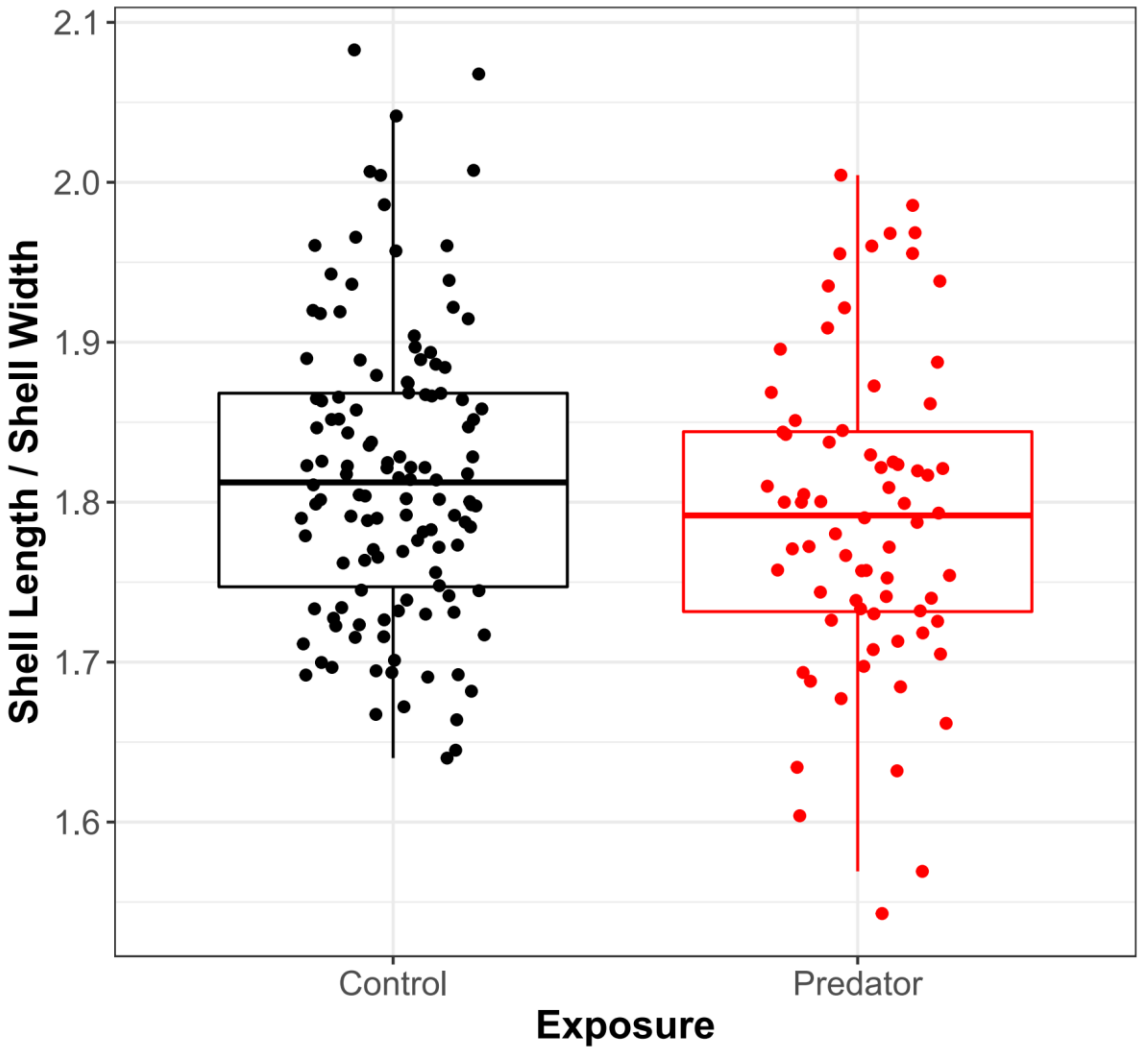

Figure 2

The proportion of shell length to shell width for F1 snails not exposed to predator cues (Control) or exposed to predator cues.

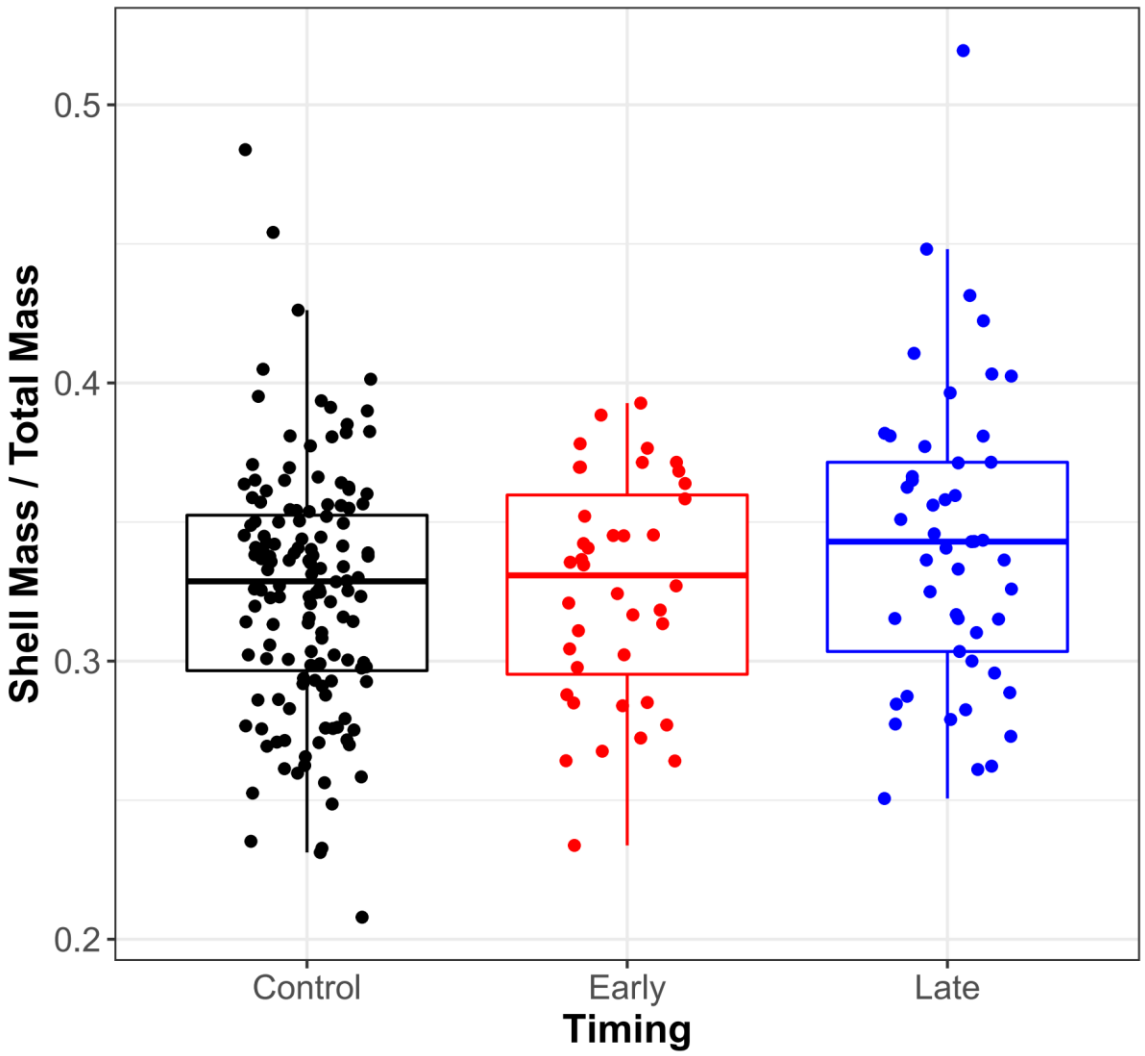

Figure 3

The proportion of total mass that is shell mass for F1 snails not exposed to predator cues (Control), exposed to predator cues early, or exposed to predator cues late.

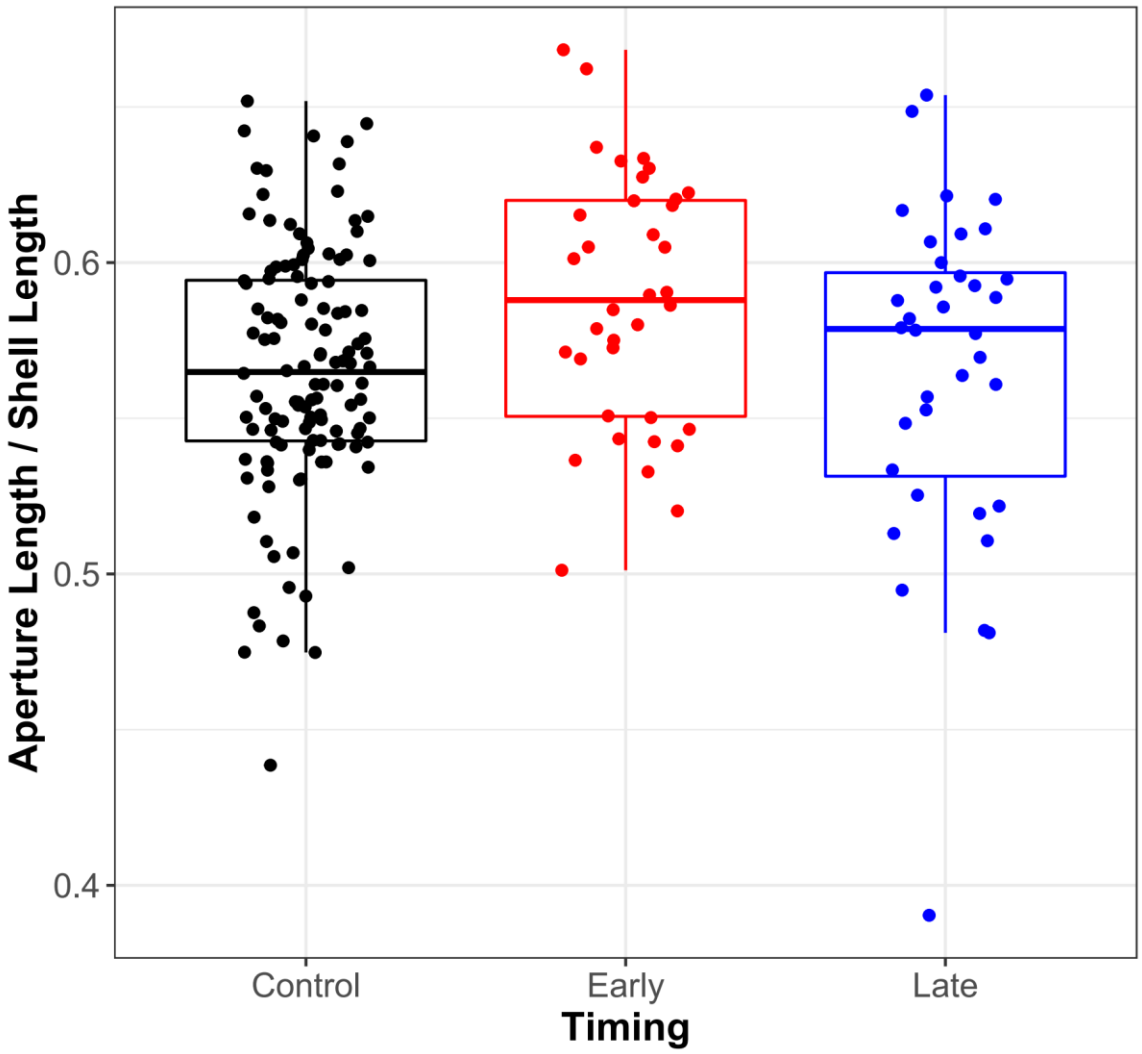

Figure 4

The proportion of aperture length to shell length for F1 snails not exposed to predator cues (Control), exposed to predator cues early, or exposed to predator cues late.

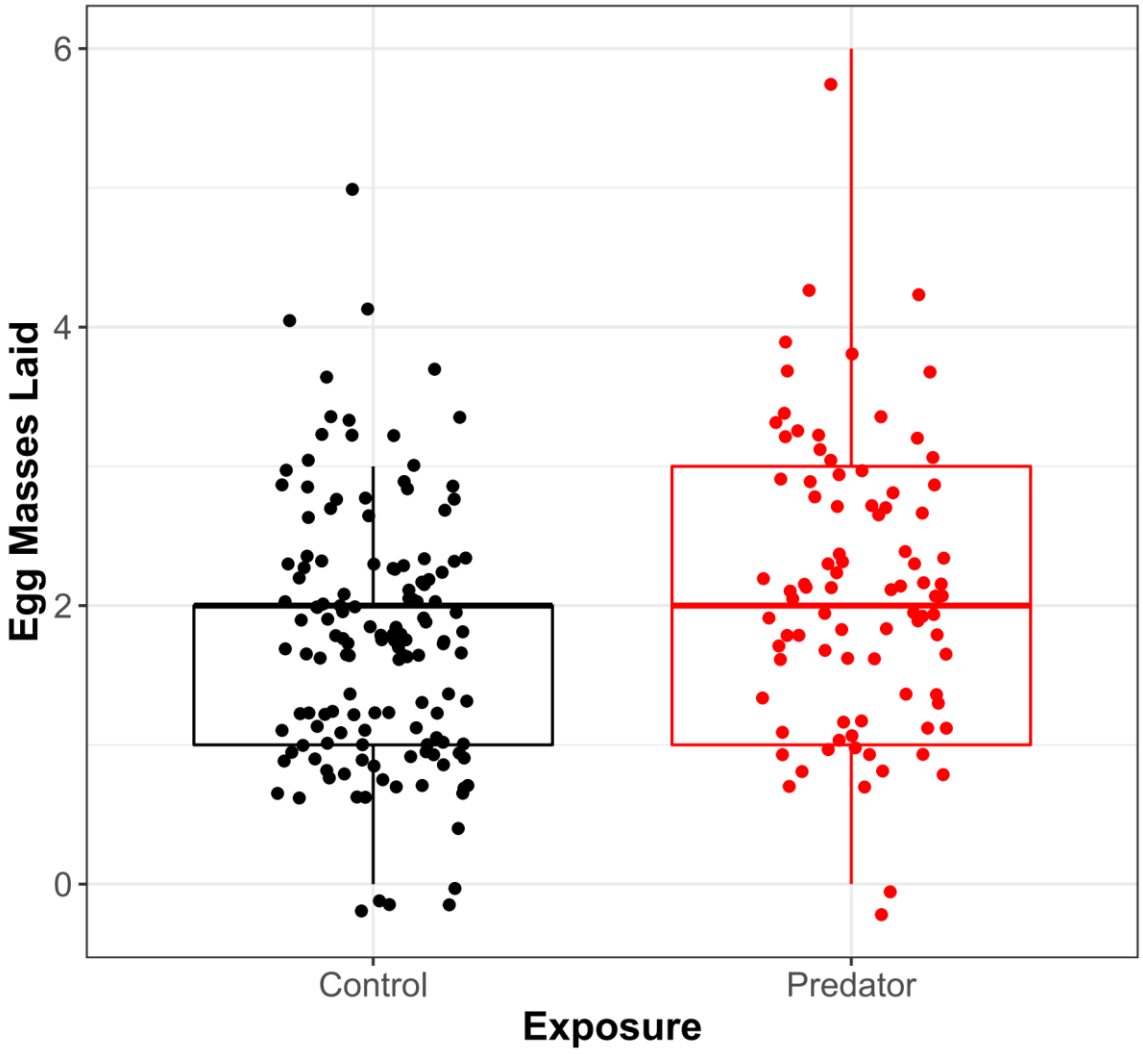

Figure 5

The total number of egg masses laid by F1 snails exposed to control cues or predator cues.

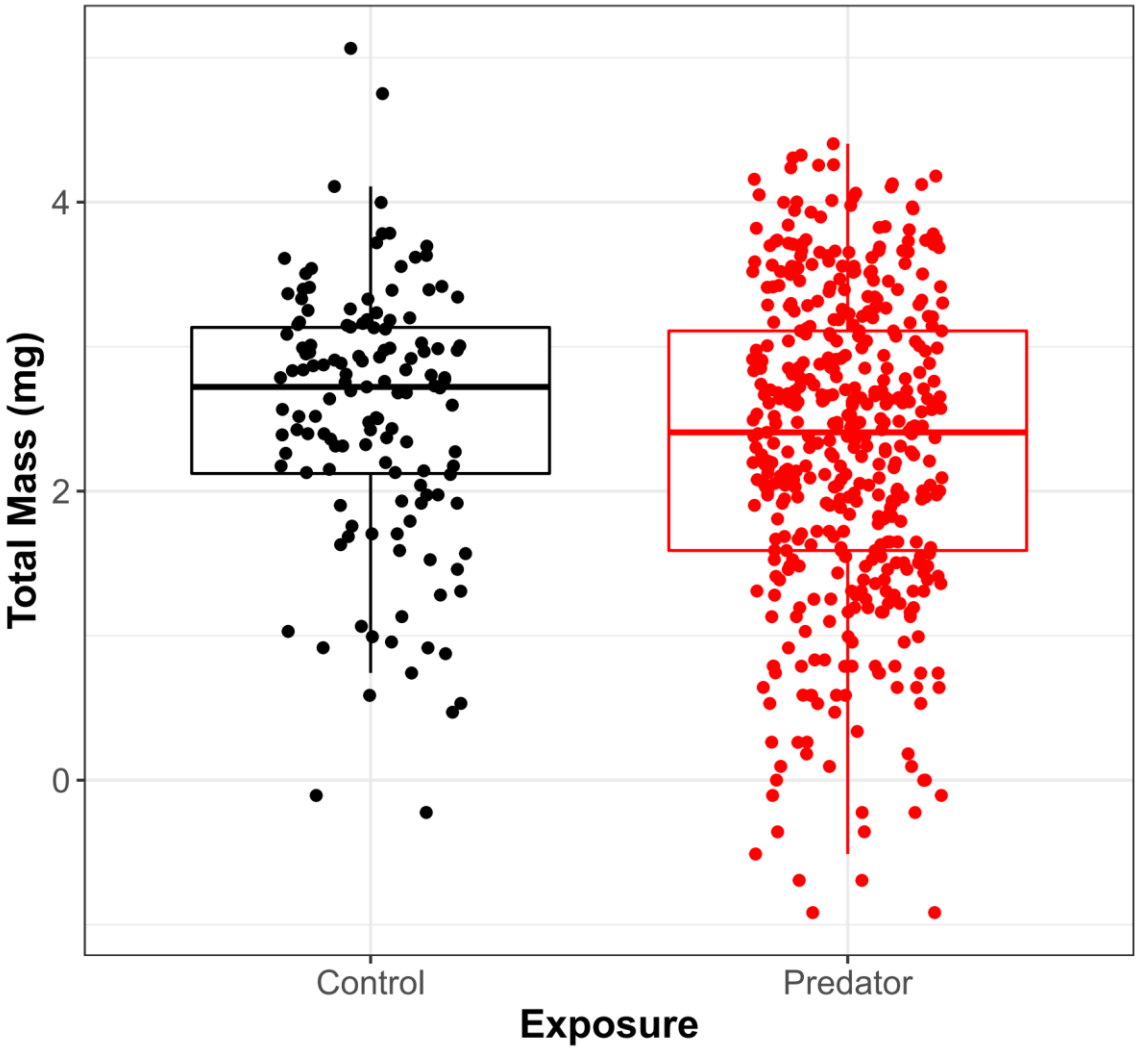

Figure 6

The total mass in milligrams of F2 snails with both parents exposed to control cues or one parent exposed to predator cues.

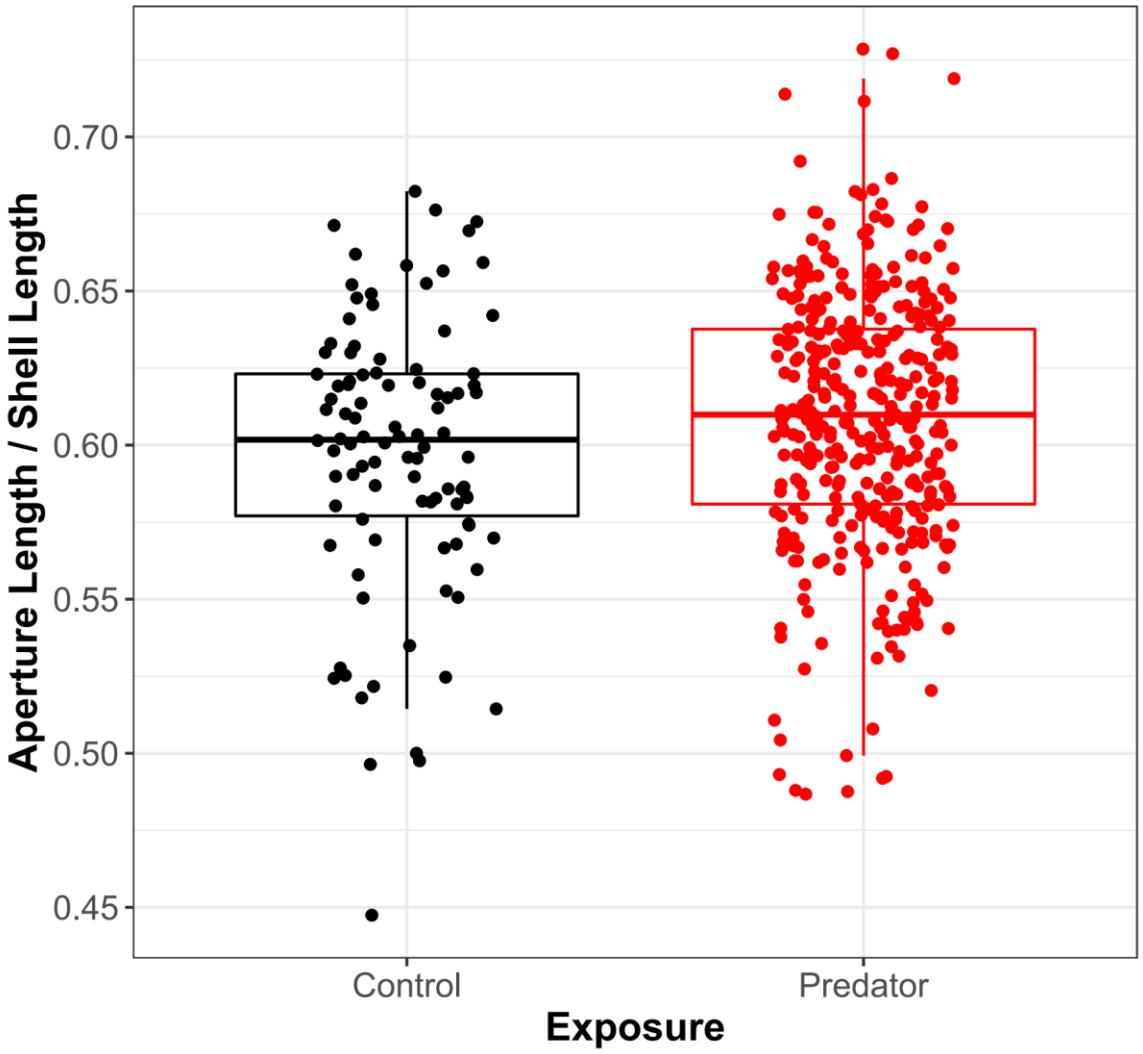

Figure 7

The proportion of aperture length to shell length for F2 snails that had parents that were not exposed to predator cues (Control), or exposed to predator cues.

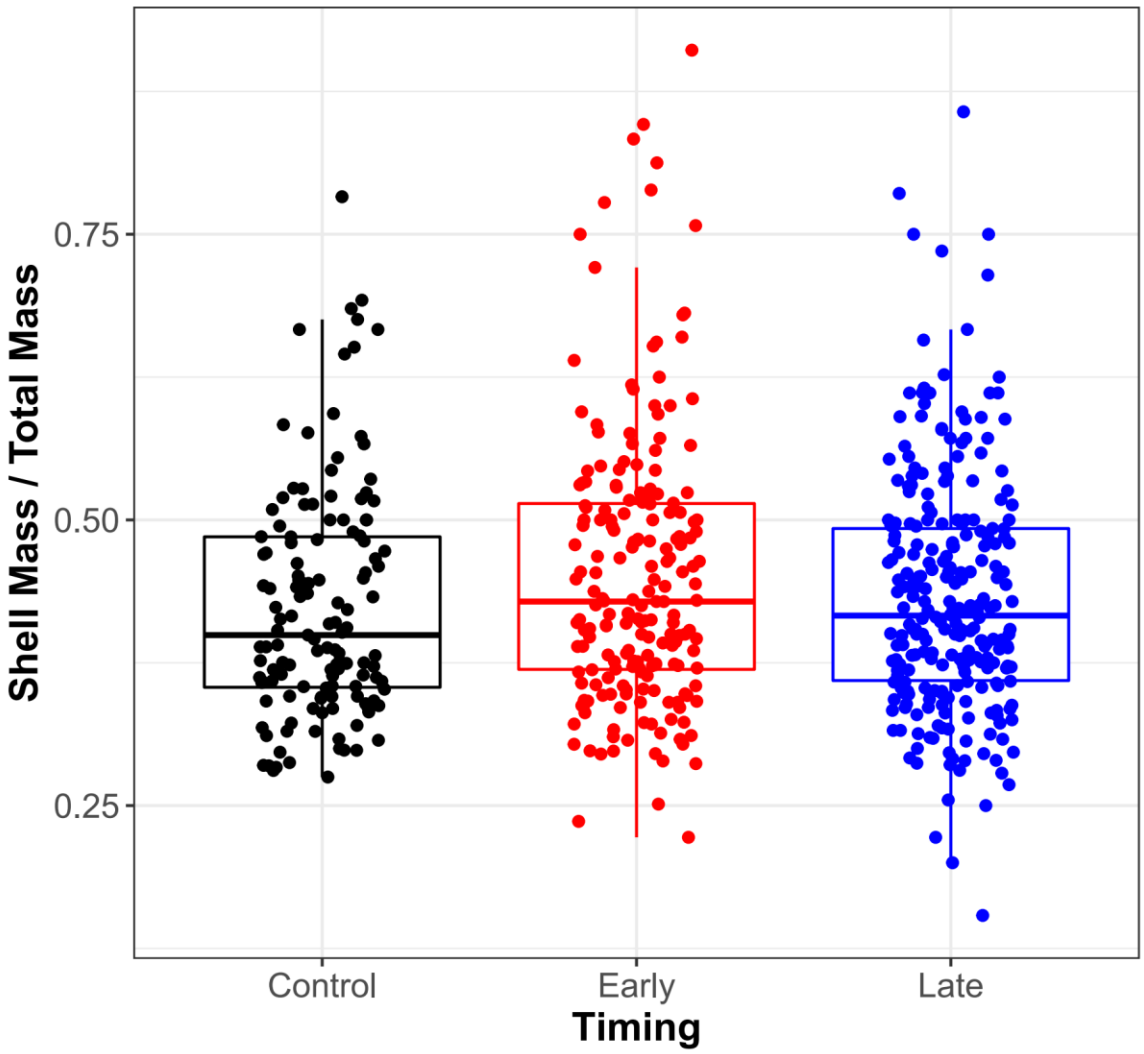

Figure 8

The proportion of total mass that is shell mass for F2 snails that had parents that were not exposed to predator cues (Control), one parent was exposed to predator cues early, or one parent was exposed to predator cues late.

VITA

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